Heterologous boost immunization with an aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) after two-dose priming with an inactivated SARS-CoV-2 vaccine in adults at 18 years of age or above: a randomized, open-label, parallel-controlled clinical trial

Protocol Number: JSVCT127

Principle Investigator: Jing-Xin Li

Sponsor: Jiangsu Provincial Center for Disease Control and Prevention

Version: 1.1

Protocol Date: August 4, 2021

Clinical Trails gov: NCT05043259

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Sponsor:	Jiangsu Provincial Center for Disease Control and Prevention	
Investigational Vaccine	Vaccine 1: Inactivated SARS-CoV-2 vaccine (Vero cells) Vaccine 2: Aerosolized Recombinant COVID-19 vaccine (Ad5 Vector)	
Protocol Date	August 4, 2021	
Version:	Version 1.1	
Principle Investigator	Jing-Xin Li	Chief physician Jiangsu Provincial Center for Disease Control and Prevention

Leading Authors

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Sponsor: Jiangsu Provincial Center for Disease Control and Prevention

Statement by Principal Investigator

I agree:

- ✧ Assume the primary investigator responsibility for this clinical study.
- ✧ Ensure that the study is carried out in accordance with the protocol and SOP in site.
- ✧ Ensure that no changes to the protocol are made without the review and written approval of the IEC, unless necessary to eliminate immediate harm to participants or to comply with regulatory requirements (e.g., administrative aspects).
- ✧ I am fully in control of the proper use of the investigational vaccines as described in the protocol.
- ✧ I am familiar with and will comply with the Good Practice for Quality Management of Drug Clinical Trials (GCP) and all relevant regulatory requirements.

Protocol Title:	Heterologous boost immunization with an aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) after two-dose priming with an inactivated SARS-CoV-2 vaccine in adults at 18 years of age or above: a randomized, open-label, parallel-controlled clinical trial
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Principle Investigator	Name: Jing-Xin Li Professional title: Chief Physician Position: Department of Vaccine Clinical Evaluation Unit: Jiangsu Provincial Center for Disease Control and Prevention Address: No. 172 Jiangsu Road, Nanjing, Jiangsu Province, China Postcode: 210009 Tel: 18915999772 fax: 025-83759529 E-mail: jingxin42102209@126.com
Principle Investigator (signature)	Date signed:

DOCUMENT HISTORY

Revision time: 2021/08/04
Original Version / Current Version: V1.0/V1.1

No.	The contents in the original version	Revised contents in the revised version	Reasons for revising
1	Investigational vaccine: 4.1 Inactivated SARS-CoV-2 vaccine (Vero cells) Manufacturer: Beijing Institute of Biological Products/Beijing Sinovac Research & Development Co., Ltd.	Investigational vaccine: 4.1 Inactivated SARS-CoV-2 vaccine (Vero cells) Manufacturer: Beijing Sinovac Research & Development Co., Ltd.	1. Revised according to the opinions of the FDA, China; 2. Revised after discussion between the sponsor and the investigators.
2	Investigational vaccine: 4.1 The aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) Specifications: 0.5ml/vial (5×10^{10}vp)	Investigational vaccine: 4.1The aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) Specifications: 1.5ml/vial (15×10^{10}vp) Using Continuous Vapouring System (manufactured by Suzhou Weiqi Biological Technology Co., LTD, Suzhou City, China) to aerosolized 0.1ml or 0.2ml of the Ad5-nCoV, and pour the droplets of vaccine into a disposable suction cup.	
3	6.1.2 Secondary endpoints: 6.1.2.1 Safety endpoints Incidence of serious adverse events (SAE) till the 6 months after booster vaccination; 6.1.2.2 Humoral immunogenicity endpoints -GMT, fold increase and seroconversion of neutralizing antibodies against live SARS-CoV-2	The safety and immunogenicity follow-up periods for participants are extended to the 12th month after boost immunization; 6.1.2 Secondary endpoints: 6.1.2.1 Safety endpoints Incidence of serious adverse events (SAE) till the 12 months after booster vaccination; 6.1.2.2 Humoral immunogenicity endpoints -GMT, fold increase and seroconversion of neutralizing antibodies against live SARS-CoV-2	

	virus at month 3, 6 after the booster dose; -GMT, fold increase and seroconversion of binding antibodies against SARS-CoV-2 RBD at month 3, 6 after the booster dose. 6.1.3 Exploratory endpoints: GMT, fold increase GMI and seroconversion positive conversion rate of anti-SARS-CoV-2 S protein SIgA antibody in saliva at 3, 6 months after the booster vaccination in immunogenic subgroups; GMT and fold increase of neutralizing antibodies against Ad5Serum anti-Ad5 adenovirus neutralizing antibody levels (GMT, GMI) at 3, 6 months after the booster vaccination in the immunogenicity subgroups;	virus at month 3, 6, and 12 after the booster dose; -GMT, fold increase and seroconversion of binding antibodies against SARS-CoV-2 RBD at month 3, 6, and 12 after the booster dose. 6.1.3 Exploratory endpoints: GMT, GMI and seroconversion positive conversion rate of anti-SARS-CoV-2 S protein SIgA antibody in saliva and IgA antibody in serum after the booster vaccination; GMT and GMI of neutralizing antibodies against SARS-CoV-2 in serum by anti-Ad5 adenovirus neutralizing antibody levels at 3, 6, and 12 months after the booster vaccination in the immunogenicity subgroups; Serum GMT of cross-neutralization test antibody levels to Delta VOCs in some participants at day 14 after the booster vaccination; Pulmonary function monitoring of the first 90 participants in the low-dose and high-dose aerosolized groups before immunization and at days 7, day 14 and day 28 after booster immunization (forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and peak expiratory flow rate (PEFR), etc.).	
4	None.	6.3 Study Plan: Add an examination of cross-neutralization antibody level for a subgroup of participants. Add an examination of lung function at visit 0 to visit 4 for a subgroup of participants.	

5	2.3 Specimen Testing Laboratories-Nanjing Vazyme Biotechnology Co., Ltd.	2.3 Specimen Testing Laboratories-Nanjing Vazyme Biotechnology Co., Ltd. is responsible for blood sample processing and ELISA antibody, and cellular immune testing; Jiangsu Center for Disease Control and prevention and the Institute for viral disease control and prevention of China Center for Disease Control and prevention are both responsible for neutralizing antibody testing (true virus).	
6	Study duration: Each subject will remain in this study for approximately 6 months from enrollment to the last visit.	The total study period is revised to 12 months; Study duration: Each subject will remain in this study for approximately 12 months from enrollment to the last visit.	
7	7.2 Inclusion criteria: No nasal or oral diseases, such as acute rhinitis (sinusitis), allergic rhinitis, oral ulcer, sore throat, etc. (Only applicable to aerosolized Ad5-nCoV group).	7.2 Inclusion criteria: No nasal or oral diseases, such as acute rhinitis (sinusitis), allergic rhinitis, oral ulcer, sore throat, etc. (for all participants).	

PROTOCOL SYNOPSIS

Brief Title:	Heterologous boost immunization with an aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) after two-dose priming with an inactivated SARS-CoV-2 vaccine in adults
Protocol Title:	Heterologous boost immunization with an aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) after two-dose priming with an inactivated SARS-CoV-2 vaccine in adults at 18 years of age or above: a randomized, open-label, parallel-controlled clinical trial
Target disease	Prevention of COVID-19 caused by SARS-CoV-2 infection
Target population	Healthy adults aged 18 years and older
Sample size	About 420 participants
Objectives	To evaluate the safety and immunogenicity of a heterologous boost immunization with an aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) after two-dose priming with an inactivated SARS-CoV-2 vaccine in adults at 18 years of age or above
Study site	Donghai Center for Disease Control and Prevention
Study Rationale	COVID-19 global pandemic has posed a serious threat to the life safety and economy of countries all over the world. Vaccines are one of the most effective ways to control the COVID-19 global pandemic. Currently, vaccines to prevent COVID-19 are mainly, inactivated vaccine, viral vectored vaccine, live attenuated vaccine, recombinant protein vaccine and nucleic acid vaccine all over the world. The inactivated vaccine produced by China Biotechnology Technology Co., Ltd. and Sinovac Life Science Co., Ltd. are prepared after inactivation and detoxification of the complete SARS-CoV-2 virus. It has shown good safety and effectiveness in clinical trials. A large phase 3 trial in Brazil showed that two doses, administered at an interval of 14 days, had an efficacy of 51% against symptomatic SARS-CoV-2 infection starting 14 days after receiving the second dose. The recombinant adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV), developed by Beijing Institute of Biotechnology (Beijing, China) and CanSino Biologics Inc, is used to express the specific S protein of COVID-19 by replication defective human adenovirus type 5 after amplification and purification. The results of the interim analysis of the Phase III clinical trial of Ad5-nCoV in Pakistan showed that 28 days after a single injection of the vaccine, efficacy of Ad5-nCoV at preventing symptomatic cases was 74.8% and 100% at preventing severe disease. Ad5-nCoV global

	phase III clinical trial data show that 28 days after a single injection of the vaccine, efficacy against severe disease was 90.98%, and the overall protective efficacy was 65.7%. Since the recombinant Ad5-vectored COVID-19 vaccine is vectored on replication defective virus particles, it can also be inoculated into the body by aerosol inhalation. Preclinical and ongoing clinical studies with the aerosol administration of Ad5-nCoV have shown that in addition to stimulating the specific immune response of the mucosal immune system to produce secretory IgA (sIgA) on the mucosal surface of the respiratory tract and serum IgA, aerosol inhalation of Ad5-nCoV also stimulates the specific immune response of the humoral and cellular immune system to produce serum IgG, interferon IFN-γ, interleukins and so on. CoronaVac (an inactivated COVID-19 vaccine) with a two-dose regimen has been authorized to use in 40 countries or areas, and been deployed globally, including China, Brazil, Malaysia, Mexico, Pakistan, Chile, Egypt, Indonesia, Nepal, and Turkey. While, one-shot regimen of Convidecia (a same recombinant Ad5-vectored COVID-19 vaccine manufactured by CanSino Biologics Inc) by intramuscular injection has been authorized to use in eight countries or areas, including China, Malaysia, Mexico, Pakistan, Chile, Ecuador, Argentina and Hungary. For inactivated vaccines, an additional dose of the heterologous or homologous vaccine may in need as part of an extended primary series. The use of a heterologous platforms vaccine for the additional dose may also be considered based on vaccine supply and access considerations. A recently published trial with a homologous boost dose of CoronaVac vaccine demonstrated a strong immune response rapidly induced by the third dose, and the neutralizing antibody titer was 3-5 times higher than that after the authorized two-dose regimen. While, another study of heterologous dose of the Ad5-nCoV Convidecia in healthy adults who have previously received inactivated COVID-19 vaccine CoronaVac, found that a heterologous boost strategy was significantly more immunogenic than a homologous boost. The combination of heterologous prime-boost schedules with inactivated vaccines and aerosolized Ad5-nCoV could potentially have a most feasible theoretical and practical application, particularly for some other low- or middle-resource countries, like China. Considering the differences in the types and characteristics of immune response between inactivated vaccines and aerosolized Ad5-vectored COVID-19 vaccine, booster vaccination of these two vaccines may form complementary advantages, improve the level of immune response and the quality of immune response, and optimize the existing immunization strategies of COVID-19 vaccine.

Investigational vaccine	**Vaccine 1:** Inactivated COVID-19 vaccine (Vero cells) **Manufacturer:** Beijing Sinovac Research & Development Co., Ltd. **Specification:** 0.5ml/ vial **Vaccine 2:** aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) **Manufacturer:** Beijing Institute of Biotechnology (Beijing, China)/CanSino Biologics Inc. **Specification:** 1.5ml/vial (15×10^{10}vp). Using Continuous Vapouring System (manufactured by Suzhou Weiqi Biological Technology Co., LTD, Suzhou City, China) to aerosolized 0.1ml or 0.2ml of the Ad5-nCoV, and pour the droplets of vaccine into a disposable suction cup. **Immunization procedure and dosages:** Three study groups are involved in this study, which are Low dose aerosolized Ad5-nCoV group, High dose aerosolized Ad5-nCoV group, Inactivated vaccine group. Recruited participants need to complete 2 injections of basic immunization with the COVID-19 inactivated vaccine. Participants in the sequential booster immunization group will be immunized with low or high dose Ad5-nCoV through aerosol inhalation in 3-9 months after the basic immunization. The low dose of aerosolized Ad5-nCoV contains 1.0×10^{10} virus particles (0.1ml), and the high dose of aerosolized Ad5-nCoV contains 2.0×10^{10} virus particles (0.2ml). Participants in the routine booster immunization group will be vaccinated with 1 dose of inactivated COVID-19 vaccine (0.5ml) in 3-9 months after the basis of immunization with 2 doses of inactivated COVID-19 vaccine. **Immunization route:** The aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) is inhaled by mouth using aerosol respiratory inhalation device; inactivated COVID-19 vaccine is injected intramuscularly into the lateral deltoid of the upper arm. **Storage and transportation conditions：** Should be stored and transported away from light under 2-8℃ and strictly prevented from freezing.
Trial design	Study design: This is a single center, randomized, open-label, parallel-controlled heterologous prime-boost immunization clinical trial. Sample size: The study groups are shown in Table 1:

Table 1. Sample size of each study group

Study group	Vaccine / dose	sample size	Interval after primary immunization	Immunogenicity subgroup
Low dose aerosolized Ad5-nCoV group	Aerosolized Ad5-nCoV/1.0×10^{10} vp (0.1ml)	140	3~9 months	The first 40 participants
High dose aerosolized Ad5-nCoV group	Aerosolized Ad5-nCoV/2.0×10^{10} vp (0.2ml)	140	3~9 months	The first 40 participants
Inactivated vaccine group	Inactivated COVID-19 vaccine /600SU (0.5ml)	140	3~9 months	The first 40 participants
Total		420		120

Note: Participants involved in this study must have completed 2 doses of inactivated vaccine immunization. The first 40 people in each group are selected to enter the expanded immunogenicity subgroup, to detect the response of cellular immune and the level of humoral immune persistence at 3, 6 and 12 months after booster immunization.

Randomization:

In the study, eligible participants will be randomly assigned to low dose aerosolized Ad5-nCoV group, high dose aerosolized Ad5-nCoV group, and inactivated vaccine group. Authorized researchers can assign the study number to the enrolled participants through the IWRS system. Researchers in vaccine management use the corresponding group of study vaccines based on the research number and grouping information.

Study plan:

This project plans to recruit 420 healthy participants aged 18 and older.

Participants who had informed consent and passed the examination and screening were randomly assigned to low dose aerosolized Ad5-nCoV group, high dose aerosolized Ad5-nCoV group, and inactivated vaccine group. Participants recruited need to complete the basic immunization with 2 doses of COVID-19 inactivated vaccine. After completing the basic immunization with 2 doses of inactivated COVID-19 vaccine, the enrolled participants will

	be boosted with 1 dose of inactivated COVID-19 vaccine or aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) in the 3 to 9 months. All enrolled participants were collected venous blood to detect serum antibody level and were collected saliva to detect secretory IgA (sIgA) level before the booster vaccination (day 0), and days 7, 14 and 28 after the booster vaccination. Among them, participants in the immunogenic subgroup need to be collected venous blood to detect serum antibody level and saliva for sIgA detection at 3, 6 and 12 months after the booster vaccination, and venous blood was collected to detect cellular immune indexes before the booster vaccination (day 0), day 7 and day 14 after the booster vaccination. For some participants, the cross-neutralization test needs to be conducted using their venous blood collected on the 14th day of immunization; about the first 90 participants in the low-dose and high-dose aerosolized groups need to undergo pulmonary function test on days 0, 7, 14 and 28. The three groups are enrolled in parallel. During the enrollment process, safety data are evaluated in real time. Once safety problems (suspension or termination criteria) of vaccination are found, later enrollment will be immediately suspended or terminated. **Study duration:** Each subject will remain in this study for approximately 12 months from enrollment to the last visit.
Endpoints	**Primary endpoints:** ● Incidence of adverse reactions within 14 days after the booster dose; ● GMT of neutralizing antibodies against live SARS-CoV-2 virus on day 14 after the booster dose; **Secondary endpoints:** ● Incidence of adverse events within 0-28 days after the booster dose; ● Incidence of serious adverse events (SAE) till the 12 months after the booster dose; ● GMT, fold increase and seroconversion of neutralizing antibodies against live SARS-CoV-2 virus on day 7 and 28 after the booster dose in each group; ● Fold increase and seroconversion of neutralizing antibodies against live SARS-CoV-2 virus on day 14 after the booster vaccination in each group; ● GMT, fold increase and seroconversion of neutralizing antibodies against live SARS-CoV-2 virus at month 3, 6, and 12 after the booster dose in immunogenic subgroups; ● GMT, fold increase and seroconversion of binding antibodies against SARS-CoV-2 RBD on days 7, 14 and 28 after the booster dose in each group;

	● GMT, fold increase and seroconversion of binding antibodies against SARS-CoV-2 RBD at month 3, 6, and 12 after the booster dose in immunogenic subgroups; ● The levels of IFN-γ、IL-2 and IL-13 secreted by specific T cells on days 7 and 14 after the booster vaccination. **Exploratory endpoints:** ● GMT, GMI and seroconversion positive conversion rate of anti-SARS-CoV-2 S protein SIgA antibody in saliva and IgA antibody in serum after the booster vaccination; ● GMT and GMI of neutralizing antibodies against SARS-CoV-2 in serum by anti-Ad5 adenovirus neutralizing antibody levels at 3, 6, and 12 months after the booster vaccination in the immunogenicity subgroups; ● Serum GMT of cross-neutralization test antibody levels to Delta VOCs in some participants at day 14 after the booster vaccination; ● GMT and fold increase of neutralizing antibodies against Ad5 at 3, 6, and 12 months after the booster vaccination in the immunogenicity subgroups; ● GMT of cross-neutralization antibody level in some participants at day 14 after the booster vaccination; ● Pulmonary function monitoring of the first 90 participants in the low-dose and high-dose aerosolized groups before immunization and at days 7, 14 and 28 after booster immunization (forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and peak expiratory flow rate (PEFR), etc.).
Scheduled site visits	Visit Plan: This study needs to complete 7 visits, as follows: V1: At day 0 (booster vaccination), informed consent and signing, physical examination, consultation screening, randomization, pulmonary function testing, pre-immunization blood and saliva collection, booster vaccination and observation are required; V2: At day 7 after the booster vaccination, safety follow-up, pulmonary function test, blood and saliva collection should be carried out; V3: At day 14 after the booster vaccination, safety follow-up, pulmonary function test, blood and saliva collection should be carried out; V4: At day 28 after the booster vaccination, safety follow-up, pulmonary function test, blood and saliva collection should be carried out; V5: At month 3 after the booster vaccination, safety follow-up, blood and saliva collection should be carried out (only in immunogenic subgroup);

V6: At month 6 after the booster vaccination, safety follow-up, blood and saliva collection should be carried out (only in immunogenic subgroup);
V7: At month 12 after the booster vaccination, safety follow-up, blood and saliva collection should be carried out (only in immunogenic subgroup);

Table2. Program and contents of visit plan of regimen participants

Visit No.	V1	V2	V3	V4	V5	V6	V7
Visit interval	Day 0	Day 7	Day 14	Day 28	Month 3	Month 6	Month 12
Time window		（+2 days）	（+3 days）	（+5 days）	（±15 days）	（±15 days）	(±30 days)
Informed consent	●						
Demographic information collection	●						
Oral and nasal disease examination	●						
Axillary body temperature measurement	●						
Urine pregnancy test (for women of childbearing age only)	●						
Inclusion and exclusion criteria screening	●						
Randomization	●						
Pulmonary function testing	●	●	●	●			

	Blood collection	●10ml (20ml) *	●10ml (20ml) *	●10ml (20ml) *	●10 ml	●10ml *	●10ml*	●10ml *
	Saliva collection	2ml	2ml	2ml	2ml	2ml*	2ml*	2ml*
	Vaccination and Observation for 30 min post-vaccination	●						
	Safety visit (AR/AE)	●	●	●	●			
	Distribution diary card (within day 14)	●						
	Return diary card (within day 14) and distribute diary card (14 days later)			●				
	Return diary card (14 days later)				●			
	Report serious adverse event (SAE)	●	●	●	●	●	●	●
	* Indicates that it is only performed in the immunogenic subgroup Note: Telephone follow-up/face-to-face interviews are conducted in the non-immunogenic subgroup at 3 months, 6 months and 12 months.							
Criteria for pausing or early termination	**Criteria for pausing:** ● Occurrence of one or more grade 4 adverse event or serious adverse event that may be associated with vaccination; ● Occurrence of grade 3 adverse events with similar symptoms associated with vaccination in 10% of participants or more. **Investigators can terminate the study when any criteria for early termination is met:**							

	● When the grade 4 adverse event or serious adverse event occurs that may probably be associated with vaccination, the principal investigator will organize a panel discussion to decide whether to terminate the study; ● When the grade 3 adverse events associated with vaccination occur in 15% of participants or more and fail to be reduced to grade 1 or 2 for more than 48 hours, the principal investigator will organize a panel discussion to decide whether to terminate the study; ● The principal investigator call for a complete termination of the trial and explain the reasons; ● Ethics committee call for a complete termination of the trial and explain the reasons; ● Administrative authority call for a complete termination of the trial and explain the reasons.
Statistical analysis	Primary analysis The safety data and immunogenicity data of the 28th day after the completion of the immunization program shall be analyzed for the first time after the results are verified to be correct. Additional analysis The safety data and immunogenicity data of the 12th month after the completion of the immunization program shall be analyzed for the second time after the results are verified to be correct. The first analysis does not consider consumption α.
Inclusion criteria and exclusion criteria	**Inclusion Criteria:** 1. Health participants aged ≥18 years, completed two doses of inactive SARS-CoV-2 vaccine in the past 3-9 months; 2. The research participants can give informed consent and sign the informed consent form (ICF); 3. The participants are able and willing to comply with the requirements of the clinical trial program and can complete the 12-month follow-up of the study; 4. No nasal or oral diseases, such as acute rhinitis (sinusitis), allergic rhinitis, oral ulcer, sore throat, etc; 5. Axillary temperature ≤ 37.0 ℃; 6. Individuals who are in good health condition at the time of enrollment, which is determined by medical history, physical examination and clinical judgment of the investigator. **Exclusion Criteria:** 1. having a medical history or family history of convulsion, epilepsy,

	encephalopathy and psychosis; 2. suffering from more serious cardiovascular diseases, such as arrhythmia, conduction block, myocardial infarction, severe and uncontrollable hypertension; 3. suffering from abnormal pulmonary function such as asthma, chronic obstructive pulmonary disease and pulmonary fibrosis; 4. being allergic to any component of the research vaccines, or having a history of hypersensitivity or serious reactions to vaccination in the past; 5. women with positive urine pregnancy test, pregnant or breast-feeding, or who have a pregnancy plan within twelve months; 6. have symptoms of upper respiratory tract infection; 7. having acute febrile diseases and infectious diseases; 8. having severe chronic diseases or condition in progress cannot be smoothly controlled, such as asthma, diabetes, thyroid disease; 9. congenital or acquired angioedema / neuroedema; 10. having the history of urticaria within 1 year before receiving the investigational vaccine; 11. having asplenia or functional asplenia; 12. having thrombocytopenia or other coagulation disorders (which may cause contraindications for intramuscular injection); 13. fainting during acupuncture (for inactivated vaccine group only); 14. having the history of immunosuppressive therapy (continuous oral administration or venous transfusion for more than 14 days), antiallergy therapy, cytotoxic therapy or inhaled corticosteroidsover the past 6 months; 15. having received blood products within 4 months before injection of investigational vaccines; 16. having received another investigational product within 1 month before injection of the investigational vaccine; 17. having received attenuated live vaccine within 1 month before injection of the investigational vaccine; 18. under anti tuberculosis treatment; 19. contrary to the trial scheme, or affecting the participants to sign the informed consent because of various medical, psychological, social or other conditions based on the judgment of the investigators.
Principle investigator	Name: Jing-xin Li Unit: Jiangsu Provincial Center for Diseases Control and Prevention Address: No. 172 Jiangsu Road, Nanjing, China

	Postcode: 210009 Tel: 18915999772 Fax: 025-83759529 E-mail: jingxin42102209@126.com
Laboratory main detection sponsor 1 (responsible for blood sample processing, ELISA antibody and cellular immune indexes detection)	Person in charge: Pan Du Unit: Nanjing Vazyme Biotechnology Co., Ltd Address: Building C1-2, Hongfeng Science and Technology Park, Kechuang Road, Economic and Technological Development District, Nanjing, China Postcode: 210000 Telephone: 13598857057 E-mail: dupan@vazyme.com
Laboratory main detection sponsor 2 (responsible for the against live virus antibody detection and cross-neutralization test in P3 laboratory)	Unit 1： Person in charge: Xi-Ling Guo Unit: Jiangsu Provincial Center for Diseases Control and Prevention Address: No.172 Jiangsu Road, Nanjing, China Postcode: 210009 Telephone: 025-83759424 E - mail: 1250535183@qq.com Unit 2: Person in charge: Wen-Jie Tan Unit: Institute of viral disease control and prevention, China Center for Disease Control and Prevention Address: No.155 Changbai Road, Changping District, Beijing Postcode:102206 Tel: 13651136235 E - mail: yanwwzz@163.com
Statistics sponsor	Person in charge: Xue-Wen Wang Unit: Shanghai Canming Medical Technology Co., Ltd Address: 6 / F, block C, No. 785 Hutai Road, Jing an District, Shanghai Postcode: 200040 Telephone: 13761517181

	E-mail: xuewen.wang@gcpdata.com

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Term/ Abbreviation	Definition/ Full Form
AE	Adverse Event
AR	Adverse Reaction
Ad5	Replication Defective Human Adenovirus Serotype 5
CDC	Center for Disease Control and Prevention
COVID-19	Corona Virus Disease 2019
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMFI	Geometric Mean of the Fold Increase
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
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SS	Safety Set
VP	Virus Particle

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1. Purpose and Introduction

There are five main technical routes for global COVID-19 vaccine research and development, which are inactivated vaccines, live attenuated vaccines, recombinant protein vaccines, viral vector vaccines, and nucleic acid vaccines. Based on the results of foreign phase III clinical trials, the inactivated vaccines of China Biotechnology Technology Co., Ltd. and Sinovac Life Science Co., Ltd., and the recombinant new coronavirus vaccine (adenovirus vector) jointly developed by the Institute of Bioengineering of the Beijing Institute of Biotechnology (Beijing, China) and CanSino Biotech Co., Ltd. had been conditionally listed. The results of the initial phase III clinical trials in foreign countries show that the short-term protection rate of inactivated COVID-19 vaccine and the Ad5 vector COVID-19 vaccine against symptomatic COVID-19 is between 50% and 70%, although it has reached the minimum 50% proposed by the World Health Organization (WHO) % Protection rate requirements, the protection rate is medium. Compared with the 95% protection rate of Moderna and Pfizer/BioNTech mRNA vaccines, there is no obvious advantage.

In order to induce the body to produce a sufficiently high level of immune response, Russia took the lead in the world to adopt a heterogeneous booster immunization program based on two different viral vector (rAd26/rAd5 vector) COVID-19 vaccines. The protection rate after sequential immunization with rAd26/rAd5 vector COVID-19 vaccines is 91.4%. The United Kingdom has also announced the launch of a heterogeneous booster vaccination study of adenovirus vector COVID-19 vaccine and mRNA vaccine, in order to optimize the immunization procedures of their respective COVID-19 vaccines and achieve better protection effects in a short period of time. In addition, with the global epidemic of SARS-CoV-2 variant strains, it has brought great challenges to the first-generation vaccine against the original strain to prevent the variant strains. The booster immunization of the second-generation COVID-19 vaccine is carried out on the basis of the first-generation COVID-19 vaccine immunization, which provide the possibility to prevent the SARS-CoV-2 variant strains.

Theoretically, the types and characteristics of the immune response induced by the Ad5 vector COVID-19 vaccine and inactivated COVID-19 vaccine are significantly different. Heterologous boost immunization with the Ad5 vector COVID-19 vaccine after priming immunization with the inactivated COVID-19 vaccine may improve the level and persistence of immune response, and optimize the existing immunization strategy of the COVID-19 vaccines.

This study intends to evaluate the safety and immunogenicity of a heterologous boost immunization with an aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) after two-dose priming with an inactivated SARS-CoV-2 vaccine in adults at 18 years of age or above. The study protocol is formulated in accordance with the requirements of the "Drug Administration Law", "Vaccine Administration

Law", "GCP", "Vaccine Clinical Trial Technical Guidelines" and "Vaccine Clinical Trial Quality Management Guidelines" (Trial).

2. Research Management

2.1 Research Institute -Jiangsu Provincial Centers for Disease Control and Prevention (Jiangsu Provincial Public Health Research Institute)

(1) Participate in the formulation of a clinical research protocol and organization of the clinical research protocol;

(2) Assist in the preparation and review of informed consent, vaccination and visit records, diary cards and other documents;

(3) Submit the ethical review materials to the Independent Ethic Committee (IEC) and obtaining the approval certificate;

(4) Prepare the Standard Operating Procedure to ensure that the research is carried out in accordance with the research plan and on-site Standard Operating Procedure;

(5) Recommend clinical research sites, and organizing and assisting in the standardized construction of sites;

(6) Have management mechanisms and measures to prevent and deal with emergencies in vaccine clinical research, and having SAE emergency response expert team and technical ability to deal with SAE;

(7) Organize on-site recruitment and enrollment of participants, organize on-site vaccination, and supervise the implementation of on-site work;

(8) Organize and ensure the safe storage and use of research vaccines, organize and manage biological samples;

(9) Organize the follow-up of participants and the collection of adverse events at the research site, and organize the report, investigation and handling of adverse events;

(10) Organize and complete all forms and Electronic Case Report Form (eCRF) entry in the study site;

(11) Confirm and manage the archived status of research materials;

(12) Issue clinical research summary report.

2.2 Research Site - Donghai County Center for Disease Control and Prevention

(1) Establish a team of on-site investigators and environmental facilities that meet the requirements of clinical research, and carry out clinical research in strict accordance with the requirements of the clinical research plan and on-site operation manual;

(2) Recruit and enroll participants who meet the requirements of the clinical research protocol;

(3) Complete vaccination, sample collection and safety follow-up observation;

(4) Handle the adverse events that occurred during the study and report serious adverse events in accordance with regulations;

(5) Collect the original data of clinical research and entering it into eCRF;
(6) Manage research vaccines and biological samples as required.

2.3 Specimen Testing Laboratories - Nanjing Vazyme Biotechnology Co., Ltd., Jiangsu Center for Disease Control and prevention, and Institute for viral disease control and prevention of China Center for Disease Control and prevention

(1) Vazyme is responsible for blood sample processing, ELISA antibody and cellular immune indexes testing; Jiangsu Center for Disease Control and prevention and the Institute for viral disease control and prevention of China Center for Disease Control and prevention are both responsible for the detection of neutralizing antibody against live virus;
(2) Complete the test of the clinical research samples according to the prescribed methods and issue a test report;
(3) Provide result judgment reference value;
(4) Issue certification, accreditation and quality control and other laboratory-related qualification certificates.

2.4 Statistical analysis company-Shanghai Canming Medical Technology Co., Ltd

(1) Participate in the formulation of clinical research plans;
(2) Write a statistical analysis plan based on the clinical research plan;
(3) Statistical analysis and issuance of statistical analysis reports.

3. Background and Principle

3.1 Pathogen

2019 Novel Coronavirus (SARS-CoV-2) belongs to the genus β of coronavirus, with enveloped granules that are round or elliptic, often pleomorphic, with diameters ranging from 60 nm to 140nm. Its genetic characteristics were significantly different from those of SARS-CoV and MERS-CoV.

SARS-CoV-2 Coronaviruses belong to the genus Coronavirus in the family Coronaviridae. Coronaviruses are single-stranded RNA viruses with an envelope. They are a large group of viruses that exist widely in nature. Globally, 10% to 30% of upper respiratory tract infections are caused by HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1, which are the second leading cause of the common cold, after rhinoviruses. Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS), caused by coronavirus, are known to be serious infectious diseases.

The coronavirus genome encodes spike protein (S), envelope protein (E), membrane protein (M) and nucleoprotein (N) in sequence. Among them, S protein is the most important surface protein of coronavirus, which is related to the transmission ability of the virus. S protein contains two subunits: S1 and S2. S1 mainly contains receptor binding region, which is responsible for the recognition of cellular receptors. S2 contains the basic elements for the membrane fusion process. In the previous development of SARS and MERS vaccines, S protein is used as the most important candidate antigen.

3.2 Disease and epidemiological background

The COVID-19 is mainly characterized by fever, dry cough and fatigue. A small number of patients have symptoms such as nasal congestion, runny nose, sore throat, myalgia and diarrhea. Severe patients usually develop dyspnea and/or hypoxemia one week after onset, and in severe cases, patients may rapidly progress to acute respiratory distress syndrome, septic shock, refractory metabolic acidosis, haemorrhagic dysfunction and multiple organ failure, etc. It is worth noting that in the course of the disease for the severe and critical patients, there may be moderate to low fever, or even no obvious fever. Some children and newborns showed atypical symptoms, such as diarrhea, vomiting and other digestive tract symptoms, or only mental weakness and shortness of breath.

At present, the source of infection is mainly patients infected by SARS-CoV-2. An asymptomatic infected person may also be a source of infection. The main route of transmission is by respiratory droplets and close contact. Exposure to high concentrations of aerosols in a relatively closed environment for a long period of time has the potential for aerosol transmission. SARS-CoV-2 can be isolated from feces and urine, and attention should be paid to the aerosol or contact transmission caused by

feces and urine to environmental pollution. The population is generally susceptible.

3.3 Basis of the study

The recombinant novel coronavirus vaccine (adenovirus type 5 vector) is developed by the Beijing Institute of Biotechnology and CanSino Biologics Inc. This vaccine is based on a mature platform of recombinant replication defective human type 5 adenovirus vector, which could efficiently express the target antigen (S protein) in transfected/infected cells. It is expected that humoral and cellular immune responses against the S protein of SARS-CoV-2 can be induced after vaccination, and provide protection to the recipients.

At present, the main kinds of novel coronavirus vaccines under research are as follows:

Inactivated vaccine: composed of a complete inactivated virus. The inactivated virus is loss of pathogenicity while maintains all or part of the immunogenicity. After vaccination, the virus antigen can stimulate the body to produce immune response and achieve protective effect. The inactivated vaccine needs to go through the following steps: the virus strains are cultured and screened on suitable cells to obtain the high titer and stable virus that represents the antigenic characteristics of the virus, and it can be used to establish a seed bank for large-scale production of vaccine in the future. The preparation of candidate vaccine through the process of culture, inactivation and purification is relatively simple, which is the traditional classical way of vaccine preparation. The main deficiency of inactivated vaccine includes two aspects: first, the study on the pathogenic mechanism and immunological mechanism of novel coronavirus is not in-depth, and the inactivated virus may carry harmful components; second, live SARS-CoV-2 culture is required to be carried out under P3 biosafety conditions at present, and the production capacity will be limited.

Recombinant subunit vaccine: made from effective antigens that can stimulate the body to produce protective immunity. The antigen is safe and guaranteed, but is generally small in size and poor in immunogenicity, and needs to be increased its immunogenicity by some new techniques and adjuvants. Development of the vaccine is long and complicated.

Adenovirus vector vaccine: The replication-defective human adenovirus type 5 with the SARS-CoV-2 antigen gene can efficiently express the target antigen of SARS-CoV-2 in transfected/infected cells, thereby allowing the body to produce corresponding humoral and cellular immunity And can provide effective protection against diseases caused by SARS-CoV-2. The vaccine uses the same adenovirus vector platform as the approved recombinant Ebola virus disease vaccine, and has a certain research and development basis.

Attenuated influenza virus vector vaccine: the vaccine is vaccinated by intranasal drip, if successfully developed, it will have a certain effect on improving the vaccination

rate. There are no reports of similar vaccines in other countries around the world.

mRNA vaccine: through in vitro synthesis of mRNA, of different antigen sequences against key targets of SARS-CoV-2 virus, and then delivered to the body, the cells in vivo are translated into antigenic proteins, thus activating the immune system and causing specific immune response. MRNA drug has the advantages of simple production, easy modification, rapid synthesis and low cost, but it has the defects of poor stability and strong immunogenicity. At present, most of the mRNA vaccine products are in the clinical stage and there are no products on the market. Among them, the most promising one is Moderna Therapeutics's mRNA-1273, a vaccine that has been tested in humans without even going through animal trials. At present, Moderna announced that its vaccine has shown neutralizing activity against multiple newly emerged COVID-19 mutant strains B.1.1.7 (first discovered in the United Kingdom) and B.1.351 (first discovered in South Africa) in vitro experiments.

3.3.1 Pre-clinical immunogenicity evaluation

3.3.1.1 Mice model (intramuscular injection + mucosal immunization)

3.3.1.1.1 Anti-S IgG binding antibody detection results

The results showed that Ad5-nCoV showed good immunogenicity by two routes of administration: anti-S IgG binding antibody peaked at day 28 after inoculation by IM, and then decreased slightly; anti-S IgG binding antibody peaked at day 28 after inoculation by IN and remained stable until day 56; in high dose group, anti-S IgG binding antibody titer of IM group was higher than that of IN group (42 days, $P < 0.0001$, 56 days $P=0.0001$). In medium and low dose groups, there was no significant difference in IgG antibody titer between two routes of administration at day 42 and day 56 after inoculation ($P > 0.05$).

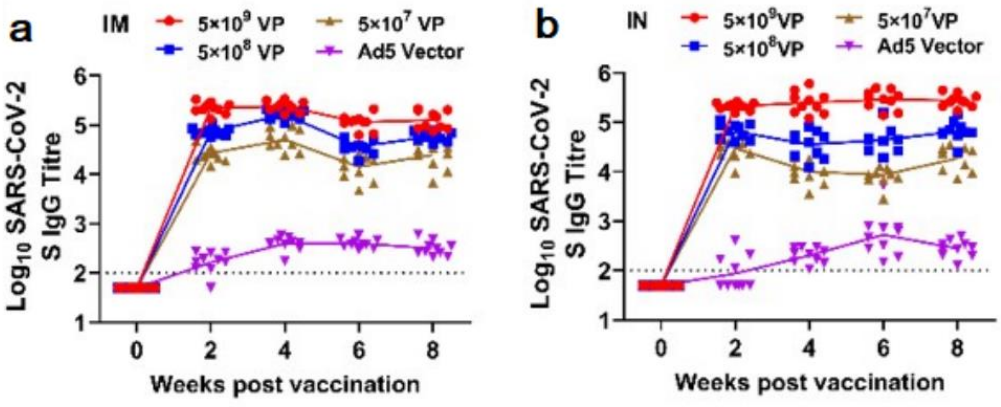

Figure 1 Levels of anti-S IgG binding antibody of mice at Day 14, Day 28, Day 42 and Day 56 after single immunization (a.intramuscular injection; b.mucosal immunization)

At 14 days and 10 weeks after immunization, anti-S IgG binding antibody was

detectable in bronchial alveolar lavage fluid in both IM and IN groups. anti-S IgA binding antibody was only detected in IN group. (**Figure 2,3**).

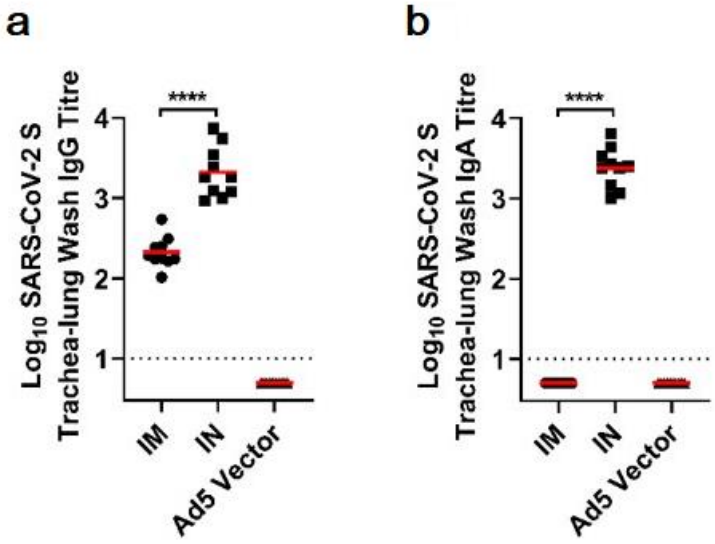

Figure 2 Levels of anti-S IgG and IgA binding antibody in serum and bronchial alveolar lavage fluid of mice after single immunization (a.trachea-lung wash IgG titre; b.trachea-lung wash IgA titre; IM:intramuscular injection; IN:mucosal injection)

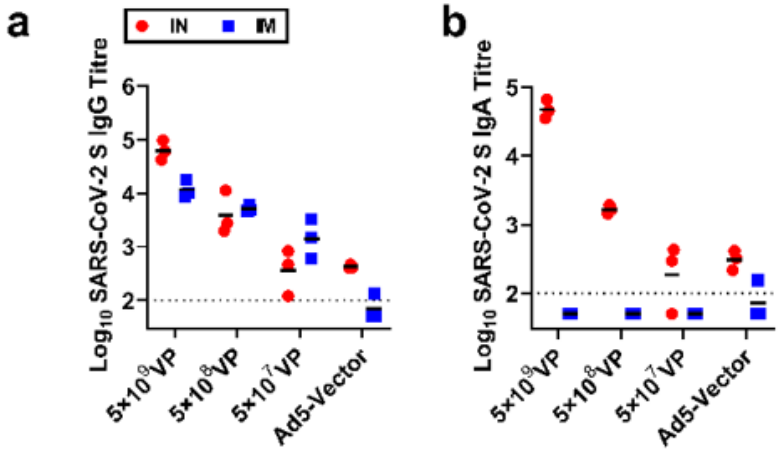

Figure 3 Levels of anti-S IgG and IgA binding antibody in bronchial alveolar lavage fluid of mice 10 weeks after immunization (a.trachea-lung wash IgG titre; b.trachea-lung wash IgA titre; IM:intramuscular injection; IN:mucosal injection)

3.3.1.1.2 Neutralizing antibody detection results

The levels of serum anti-SARS-CoV-2 neutralizing antibody (NAb) were measured by the virus-specific micro-cytopathic effect neutralization test at Day 14, Day 28, Day 42 and Day 56 after single intramuscular injection and mucosal immunization (**Figure 4**).

The results showed that Ad5-nCoV showed good immunogenicity by two routes of administration: neutralizing antibody peaked at Day 42 after inoculation by mucosal

immunization, while peaked at Day 56 after inoculation by intramuscular injection; in high dose group, neutralizing antibody titer by mucosal immunization was significantly higher than that by intramuscular injection from Day 28 to Day 56 after inoculation (Day 28, P<0.0001; Day 42, P<0.0001; Day 56, P=0.0021). There was no significant difference in neutralizing antibody titer between two routes of administration in medium dose group at Day 42 and Day 56 after inoculation. No significant differences were found in neutralizing antibody titer between two routes of administration in low dose group at each time point after inoculation.

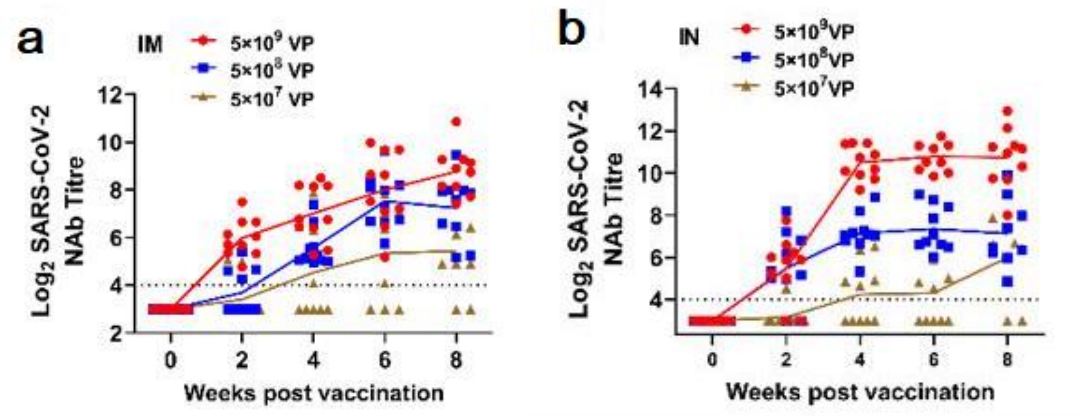

Figure 4 Levels of SARS-CoV-2 neutralizing antibody at Day 14, Day 28, Day 42, and Day 56 after single immunization in mice models (a.intramuscular injection; b.mucosal injection)

Neutralizing antibody measured by pseudo-neutralization assay were detected at 14 days,28 days and 56 days after single inoculation by IM or IN (**Figure 5**): levels of neutralizing antibody measured by wild-type assay and pseudo-neutralization assay were comparable.

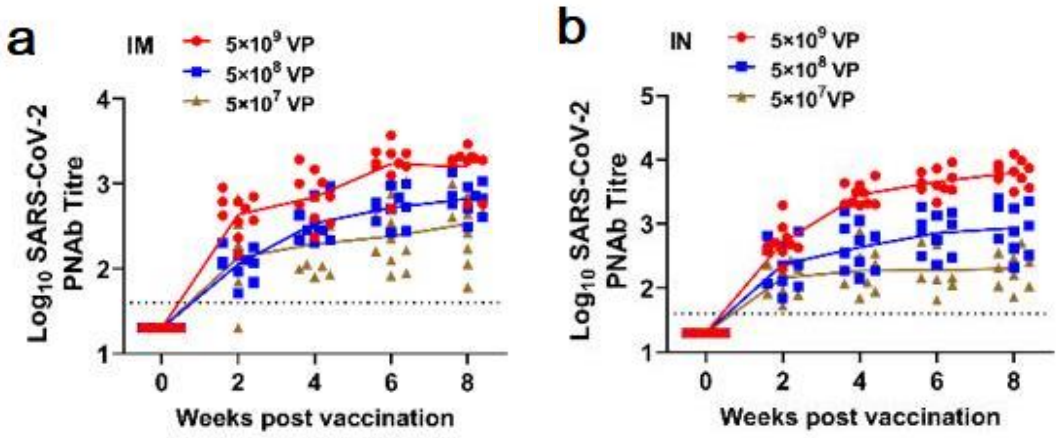

Figure 5 Results of neutralizing antibody against pseudovirus in mice at days of 14, 28, 42 and 56 after single immunization (a.intramuscular injection; b.mucosal injection)

Anti-S IgG binding antibody response correlated strongly with neutralizing antibody measured by wild-type assay and pseudo-neutralization assay at Day42 and Day56 after inoculation.

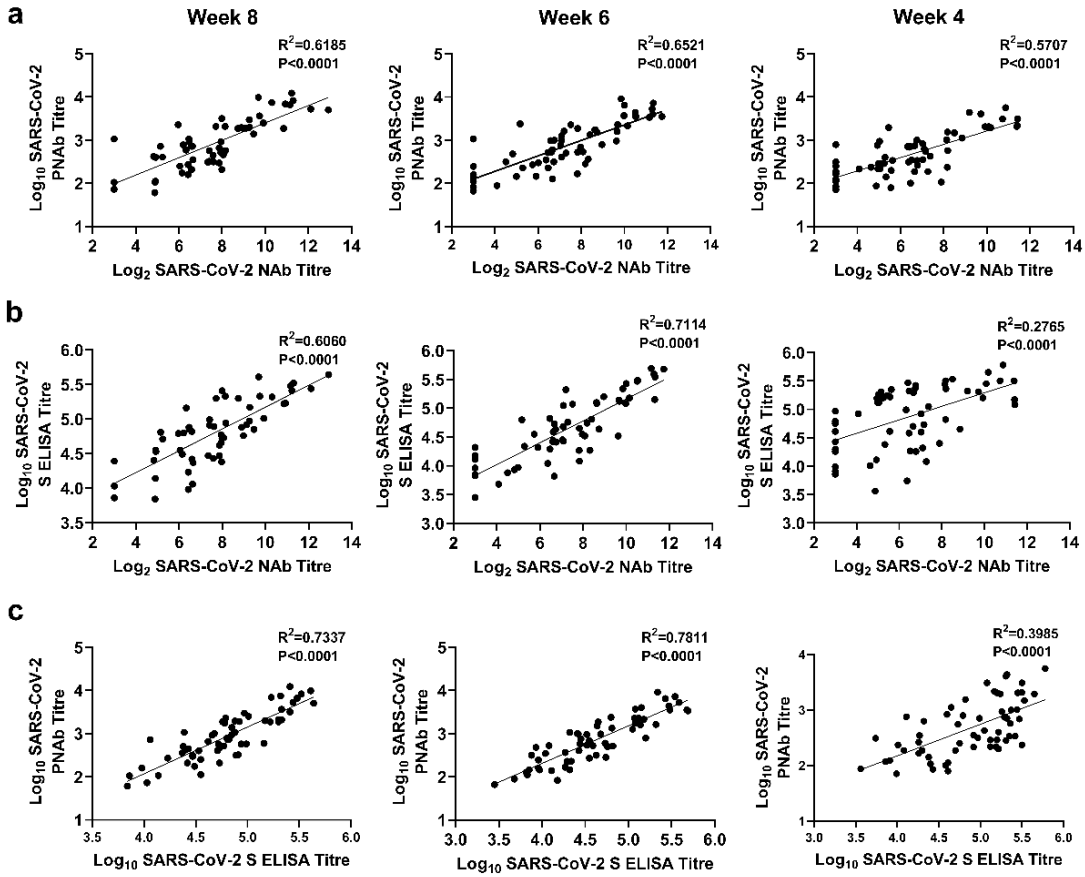

Figure 6 Correlations among IgG binding antibody, neutralizing antibody and neutralizing antibody measured by pseudo-neutralization assay in mice models immunized with Ad5-nCoV

3.3.1.1.3 Cellular Immune Response

The percentage of cytokine-positive cells in CD8+ or CD4+ T cells was measured by intracellular cytokine staining assays.

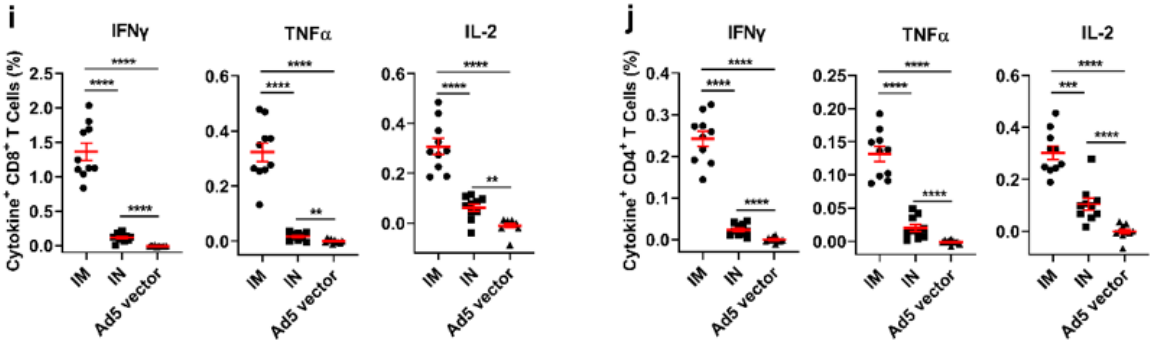

Figure 7 Cellular immune response 14 days after single immunization in mice models

In medium dose group, both intramuscular injection and mucosal immunization could significantly induce the production of IFN-γ, TNF-α and IL-2 by CD8 + and CD4 + T cells 14 days after immunization, which was higher in the intramuscular injection group than in the mucosal immunization group.

There was a dose-dependent cellular immune response in the intramuscular injection group 10 weeks after immunization. No such response was found in mucosal immunization group.

3.3.1.2 Ferret model (intramuscular injection + mucosal immunization)

3.3.1.2.1 Anti-S IgG binding antibody & neutralizing antibody detection results

18 ferrets were randomly divided into three groups: intramuscular injection group, nasal drip group and negative control, 6 in each group. The anti-S IgG binding antibody and neutralizing antibody were detected in all vaccinated groups at 28 days after immunization, but not in the control group. There was no significant difference between the two routes of administration.

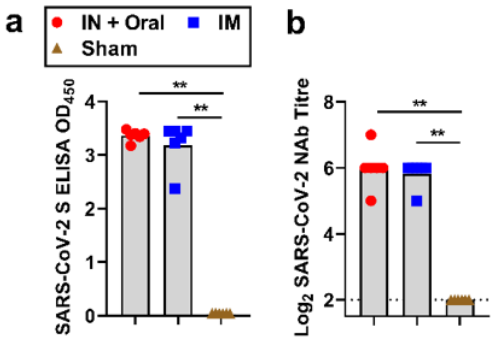

Figure 8 Levels of anti-S IgG binding antibody and neutralizing antibody in ferret models.

3.3.1.2.2 Cellular Immune Response

IFN-γ-producing T cell response (ELISpot) were detectable in 5 ferrets of intramuscular group and 3 ferrets of mucosal immunization group at 28 days after immunization.

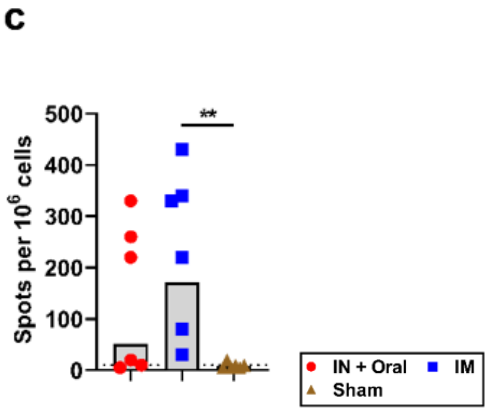

Figure 9 Cellular immune response in ferrets

3.3.1.3 Rhesus monkey model (aerosol inhalation)

3.3.1.3.1 Immunogenicity of different doses

In the "Immunogenicity study of rhesus monkey nebulized immunization with injection of different times and doses" commissioned by Beijing Zhaoyan Company, the nebulized immunization method was the same as the clinically planned aerosol inhalation route. The doses were 1 or 2 doses / person (2.5×10^{10} vp) and 3 doses / person (15×10^{10} vp), respectively, with two times of aerosol immunization, and two weeks apart. The levels of serum antibody were measured before and after each immunization. The levels of serum antibody of the first and second immunizations were measured by ELISA, and the JMP was used for statistical analysis. The results showed that for rhesus monkeys, the antibody level of 3HD was higher than 1/2HD when immunized with 1 and 2 injections.

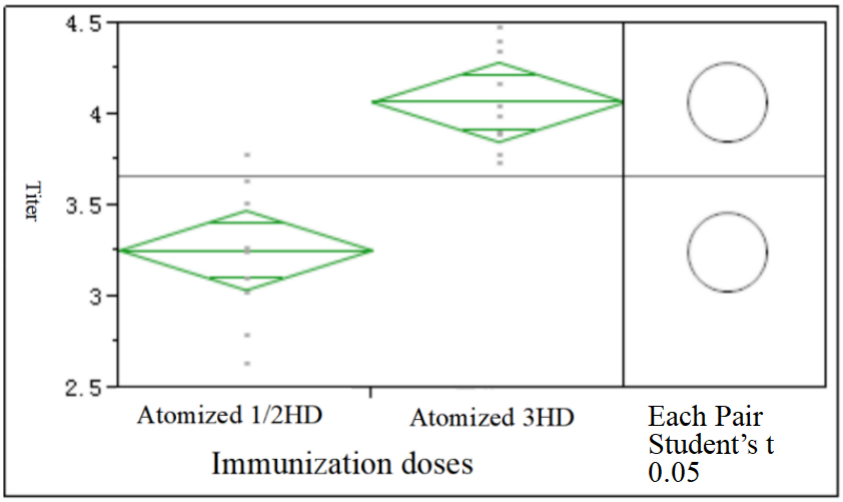

Figure 10 Serum antibody titers of different aerosol immunization with first immunization

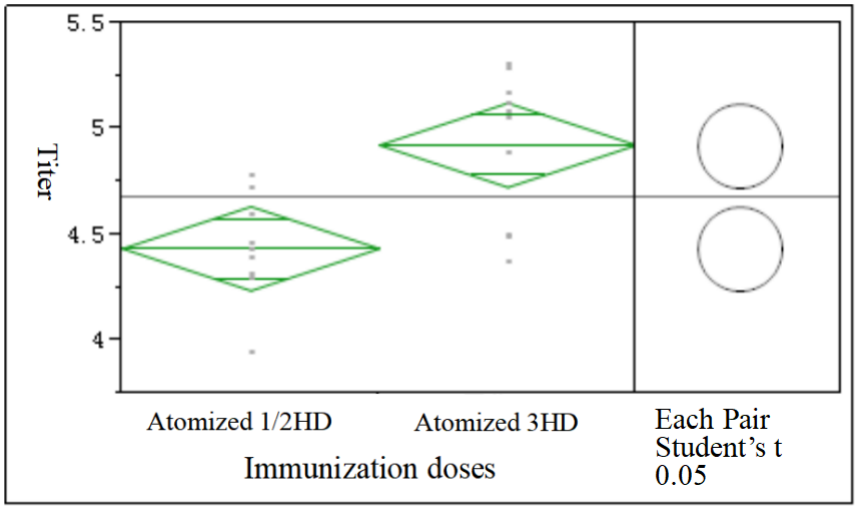

Figure 11 Serum antibody titers of different aerosol immunization with second immunization

3.3.1.3.2 Immunogenicity of different injections

Nebulized inhalation of 1/2HD test substance, immunized three times, two weeks apart. The levels of serum antibody were measured before and after each immunization. The levels of serum antibody of the first, second and third immunizations were measured by ELISA. The results showed that the levels of serum antibody of 2-doses nebulized immunization was significantly higher than that of 1-dose, and the difference was statistically significant, but the difference between 3-doses and 2-doses immunization was not significant.

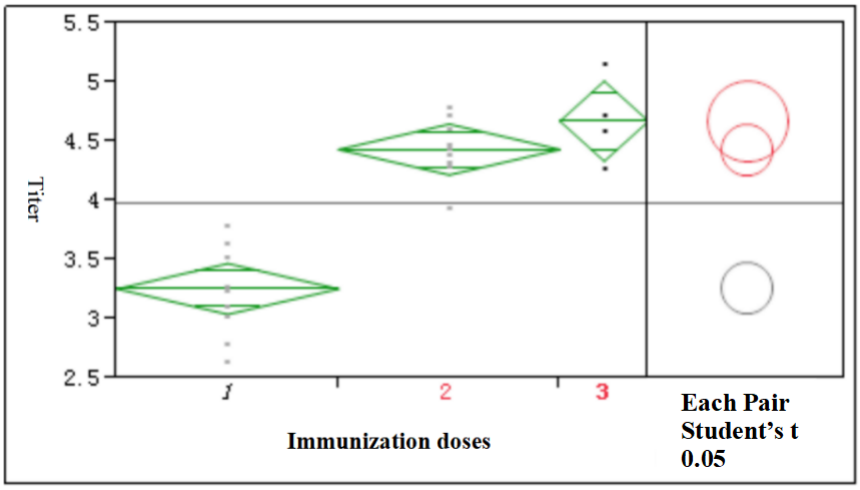

Figure 12 Serum antibody titers in the rhesus monkeys with different immunization shots

3.3.1.4 Study on the protective effect in BALB/c mice model (intramuscular injection + mucosal immunization)

For mucosal immunization group, no virus was detected in the lung and turbinate of mice euthanized 3 or 5 days after challenge. While in mice euthanized 3 days after challenge in control group, viral load was detectable, with 1.2×10^4 PFU/g in turbinate

and 5.6×10^5 PFU/g in lung on average. No virus was detectable in the lung of IM group, while the average viral load was 3.3×10^6 PFU / g in the lung of control group at day 3 after challenge. Compared with the control group, the viral load in the turbinate of mice inoculated by intramuscular was significantly reduced.

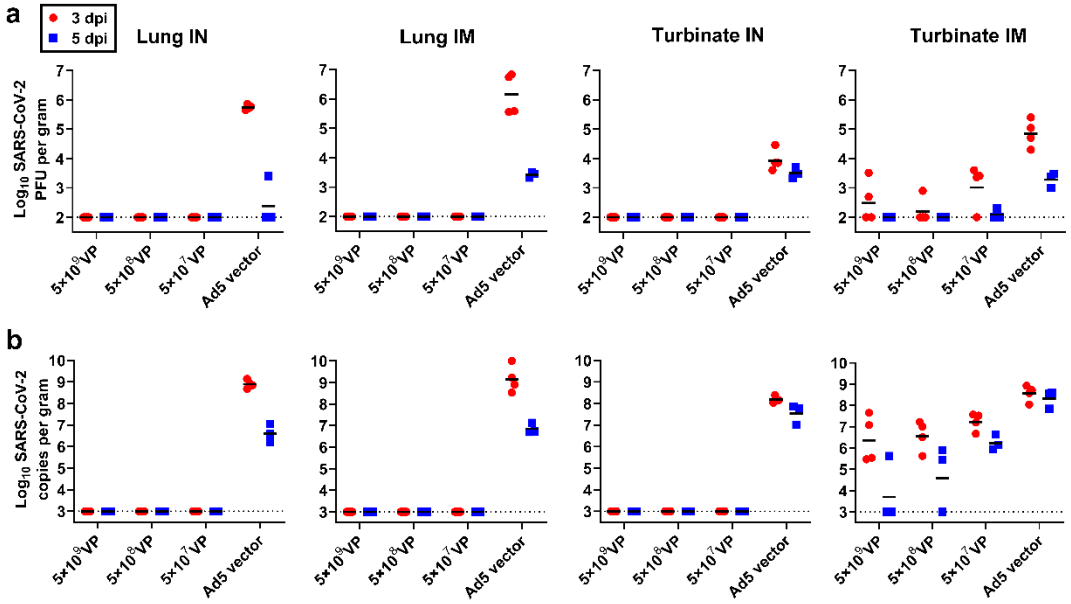

Figure 13 Number of live virus (a) and viral load (b) in lung and turbinate of mice at 3 (3 dpi) and 5 days (5 dpi) after challenge

3.3.1.5 Study on the protective effect in ferret model (intramuscular injection + mucosal immunization)

For the ferret model, the recombinant COVID-19 vaccine induced a strong serum IgG antibody and neutralizing antibody response by musculcular injection or intranasal instillation immunity, and both SARS-CoV-2 inactivated virus-specific IFN-γ were detected in peripheral blood monocytes. A 105 PFU SARS-CoV-2/HRB25 isolate challenge was performed 28 days after immunization, where complete protection was achieved in the mucosal immunization group and no virus was detected in the nasal lavage fluid of mucosal immunization group at 2 ~ 8 days after challenge. The virus was detected in some ferrets in the muscular injection group, but the number of virus was significantly reduced at each detection point compared with the control group.

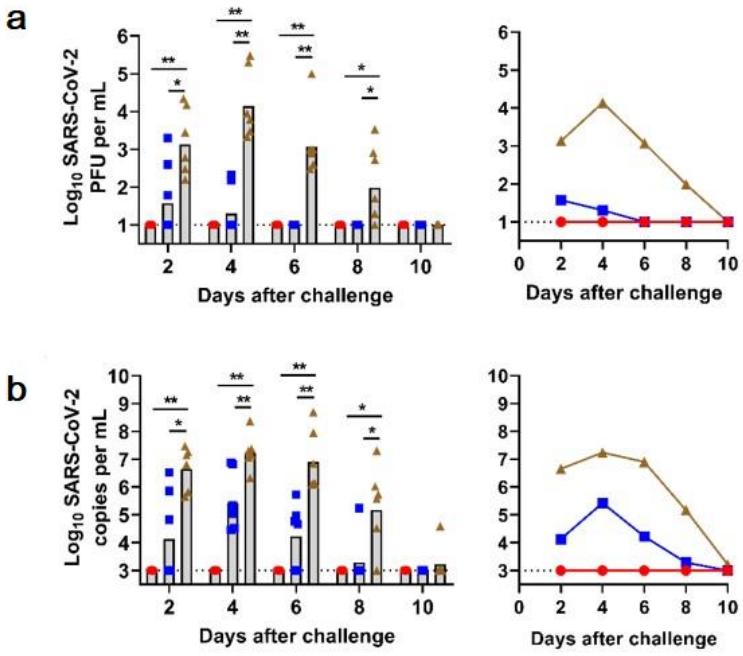

Figure 14 The number of live SARS-CoV-2 virus (a) and viral load (b) in the nasal lavage fluid of ferrets after challenge.

3.3.2 Pre-clinical toxicity study

3.3.2.1 Single-dose toxicity study by IM in SD rats

In this study, SD rats were administered with single dose of Ad5-nCoV (0.5 x10^{11} vp) by IM. No signs of toxicity were observed. The maximum tolerated dose (MTD) was greater than or equal to 0.5 x10^{11} vp/ dose/rat. IgG antibody against adenovirus vector and anti-S IgG binding antibody were detectable in 2 groups at 2 weeks after administration.

3.3.2.2 Repeated dose toxicity study by IM in rhesus monkeys (mucosal immunization, aerosol inhalation)

Ad5-nCoV repeated aerosol inhalation or intranasal instillation to rhesus monkeys for 4-week administration and 2-weeks recovery toxicity test:

No death or dying were observed during the study. No adverse clinical signs were related with the vaccine. No allergic reactions were observed in clinical observation after three doses of vaccine. Compared with the same sex negative control, no obvious changes or toxicological abnormal reaction were observed by different routes of administration in the following indicators: body weight and weight gain, body temperature, ophthalmic examination, clinical pathology (blood cell count, coagulation function, blood biochemistry, urine analysis), T lymphocyte subsets (CD3+, CD3+CD4+, CD3+CD8+, CD3+CD4+/CD3+CD8+), serum cytokines (IL-2, IL-4, IL-5, IL-6, TNF-α, IFN-γ), C-reactive protein, serum complement (C3, C4), organ weight, visceral body ratio and visceral brain ratio. Before and after the first and last

administration, no obvious changes or toxicological abnormal reaction were observed in the following safety pharmacological indicators: ECG waveform and parameters, blood pressure (systolic blood pressure, diastolic blood pressure, mean arterial pressure) and respiratory function (respiratory rate and tidal volume).

Immunogenicity data showed that, anti-S protein IgG specific antibodies were negative 10/10 animals in negative control. Transient specific IgG antibody against adenovirus vector was detected in only 1/10 animals at day 14 (D14). In low and high dose for aerosol inhalation, and high dose for intranasal instillation, only a few animals produced induced weaker specific IgG antibody against adenovirus vector with titer ranging from 1:100 to 1:200 at week 2 after first administration. No obvious changes or titers of specific IgG antibody against adenovirus vector was detectable with titer ranging from 1:100 to 1:400 by the end of the recovery period (D43). In low and high dose for aerosol inhalation, and high dose for intranasal instillation, all animals induced anti-S protein specific IgG antibodies, and antibody positive rate was 10/10 at week 2 after first administration. The range of antibody titers increased with the times of administration. The antibody titer ranged from 8721.886 to 201992.795 in the aerosol inhalation group, and from 579.049 to 10487.362 in the intranasal instillation before the last dose (D28). No antibody titers in each group declined after 2 weeks of withdrawal. The antibody titer ranged from 18154.480 to 227215.309 in the aerosol inhalation group, and from 1051.386 to 15979.091 in the intranasal instillation.

The result of antinuclear antibody showed that no antinuclear antibody was detected in negative control group and with different routes of administration during the study.

The result of pathological examination showed that no gross and histopathological changes were observed in each group at the end of the administration (D32) and the end of the recovery period (D43). No irritation responses to the local administration were observed.

Conclusion: Under the condition of this experiment, rhesus monkeys were administered with repeated Ad5-nCoV at the dose of 2.5×10^{10} vp/dose (1 dose /monkey) and 1.5×10^{11} vp/dose (1 dose /monkey) by spray inhalation or repeated at the dose of 1×10^{11} vp/dose (1 dose /monkey), once every 2 weeks for a total of 3 doses. No toxic reactions of animals were observed in each group during the study, that is, no adverse reaction dose (NOAEL) was 1.5×10^{11} vp /dose (1 dose /monkey) and 1×10^{11} vp /dose (1 dose /monkey). Two weeks after administration (D14), all the animals induced strong anti-S protein IgG specific antibody, and the antibody titer showed an increasing trend with the increase in the times of administrations; no immunotoxic reactions of animals were observed; no irritant reaction were observed at the local administration.

3.4 Previous clinical study

Phase I clinical study

A phase I study of recombinant novel recombinant coronavirus vaccine (adenovirus vector) was launched in Wuhan in March 16, 2020. Three groups of low, medium and high dose with 36 participants enrolled in each group were immunized with the candidate vaccine. Three doses of 5×10^{10} vp, 1×10^{11} vp or 1.5×10^{11} vp were used in the study.

Safety data collected at 28 days after vaccination showed that, the overall incidence rates of adverse reactions in low, medium and high dose groups were 83.33%, 83.33% and 75.00%, respectively. The incidence rate of grade 3 adverse reactions was 5.56%, 5.56% and 16.67% in three groups. Neither grade 4 adverse reaction nor serious adverse event was observed. The most common local adverse reaction was pain, and the most common systemic adverse reaction was fever. The recombinant novel coronavirus vaccine (adenovirus vector) was safe in low and medium dose groups and showed a clinically tolerable safety in high dose group.

Immunogenicity data showed that, for the neutralizing antibody measured by wild-type assay: the seroconversion rate, GMT and GMI collected at 28 days after vaccination were higher than those collected at 14 days after vaccination in low, medium and high dose groups. On the 28th day, there was no statistical difference in the seroconversion rate, GMT and GMI between the low dose and the medium dose group; while, seroconversion rate, GMT and GMI between low and high dose, or between medium and high dose were statistically different. The humoral immune response induced by high dose was better than that induced by low and medium dose.

Cellular immune response after vaccination: levels of IFN-γ collected at 14 days after vaccination measured by ELISpot were obviously higher than that collected at 28 days after vaccination. There was no significant difference in the levels of IFN-γ measured by ELISpot on the 14th day after vaccination between the medium and high dose groups; the level of IFN - γ measured by ELISpot on the 14th day after vaccination in the low dose group was significantly lower than that in the medium or high dose group, with statistically difference; the levels of IL-2, IFN - γ and TNF α expressed by CD4 + and CD8 + T lymphocytes also had similar character.

In conclusion, Ad5-nCoV vaccine was safe and immunogenic in healthy adults. The specific humoral and cellular immune responses against SARS-CoV-2 peaked at 28 days and 14 days after vaccination, respectively.

Phase II clinical study

Based on the results of phase I clinical study, medium and low dose of vaccine were selected to enter phase II clinical study, which was launched in Wuhan, China in April 12, 2020. It was a randomized, double-blind and placebo-controlled study. 508 healthy adults aged 18 years and above were randomized into low dose（5×10^{10} vp）, medium dose（1×10^{11} vp） and placebo at the ratio of 2:1:1. Safety data was showed as below:

The overall incidence rates of adverse reaction collected within 7 days and 14 days after vaccination in the low, medium dose group and placebo were 74.42%, 74.70% and 37.30%, respectively. There was no statistical difference between low dose group and medium dose group. The incidence rate of adverse reaction in low and medium dose group were significantly higher than that in placebo. The incidence rate of grade 3 adverse reaction was 0.78%, 9.49% and 0% in low, medium dose group and placebo. Neither grade 4 adverse reaction nor serious adverse event was observed. The most common local adverse reactions were injection site pain, and the most common systemic adverse reactions were fatigue, headache and fever. The recombinant novel coronavirus vaccine (adenovirus vector) was safe in low and medium dose groups, with better safety in low dose group.

Immunogenicity data showed that, the seroconversion rates of low, medium dose groups and placebo were significantly different, with seroconversion rates obviously higher in low and medium groups than placebo. The seroconversion rate of anti-S RBD binding antibody was relatively high in low and middle dose groups. On the 28th day of vaccination, the seroconversion rate of neutralizing antibody measured by pseudo-neutralization assay in low and medium dose groups was significantly higher than that in placebo.

Cellular immunity data showed that, the seroconversion rate of IFN-γ measured by ELISpot in low and medium dose groups was significantly higher than that in placebo. The recombinant novel coronavirus vaccine (Adenoavirus vector) with low dose and medium dose showed good and similar immunogenicity in human. The recombinant novel coronavirus vaccine (adenovirus vector) was immunogenic in low and medium dose groups, with similar immunogeniciy character in both groups.

Phase III clinical study

The key overseas phase III clinical trial of this product adopts a multi-center, randomized, double-blind, placebo parallel-controlled design, and was launched in Pakistan, Mexico, Russia, Chile, and Argentina in five countries 18 years and older in September 2020. It was used to evaluate the protective effect of this product. As of the interim analysis, a total of 34385 participants were randomly enrolled, of which 9.90% were participants 60 years and older.

The safety results showed that:

After being vaccinated with this product in 17,190 participants aged 18 and older in the investigational vaccine group, SAE and MAE were collected. For SAEs observed in clinical trials, investigators judged that they were not related to vaccination or may not be related. In the vaccine group, 39 cases (0.227%) had vaccination-related MAE, and there was no MAE of grade 3 or above; 42 cases (0.244%) in the placebo group had vaccination-related MAE. Among them, the incidence of pain, fever, pectoralgia, chills, pain at the inoculation site, fever at the inoculation site, reaction at the

inoculation site, abnormal sensation, dizziness, lymph node pain, lymphadenopathy, tachycardia, oral ulcer and limb pain in the experimental group were higher than those in the placebo group. As of February 5, 2021, 335 participants in the safety extended cohort had completed the 28 day systemic safety follow-up after immunization. Within 28 days after vaccination in the vaccine group and placebo group, the main adverse reactions were solicitation (68.96% vs. 48.24%), among which the main adverse reactions at the vaccination site were pain (57.61% vs. 21.76%), and the main systemic adverse reactions were headache (41.19% vs. 30.00%), myalgia (40.00% vs. 23.82%) and lethargy (31.04% vs. 21.47%). Epistaxis (0.30%) was a newly observed symptom of non-solicited adverse reactions in the experimental group.

The results of protection effectiveness showed that:

In the interim analysis, 100 valid cases were obtained for the primary study endpoint. The efficacy of Ad5-nCoV vaccine against COVID-19 28 days and later after 1 dose of immunization: The efficacy of all symptomatic cases was 65.28% (95% CI: 45.73, 77.79), which met the primary hypothesis; the efficacy of severe cases is 90.07% (95% CI: 22.41, 98.73). 205 valid cases were obtained for secondary endpoints in the interim analysis. The efficacy of Ad5-nCoV vaccine against COVID-19 14 days and later after 1 dose of immunization: The efficacy of all symptomatic cases was 68.83% (95%CI：57.03, 77.39), which met secondary hypothesis; the efficacy of severe cases was 95.47% (95%CI：66.39, 99.39).

The results of serum cross neutralization showed that, serum from 20 participants aged 18 to 55 years old 28 days after vaccination of Ad5-nCoV were used to detect neutralizing antibodies against 10 COVID-19 strains (HB01, HB02, CQ01, QD01, BJ01, DL01, XT01, SJZ01, BJ2021, GDPCC) by the micro-cytopathic method. The results showed that the GMT of neutralizing antibody against BJ2021 (British strain) was 12.38, the GMT of neutralizing antibody against GDPCC strain (South African strain) was 4.05, and GMT of neutralizing antibody against the remaining 8 strains were from 11.65 to 28.35.

Phase I and II clinical study of aerosol inhalation

In the phase I/II clinical trials of aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV), as of July 1, 2021, the safety monitoring results of 120 people blinded in phase I 0-28 days after the first injection showed that nebulized inhalation of different doses of test vaccines was safe.

4. Investigational vaccines

4.1 The aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV)

The aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) is a kind of human adenovirus 5 with replication defect which expresses SARS-COV-2 protein, used to prevent the disease caused by SARS-COV-2 infection. The

HEK293SF-3F6 cell is used for Ad5 cultivation. After amplification and purification, the vaccine liquid is made by adding appropriate excipients.

Active ingredient: recombinant replicated-defective human adenovirus 5 expressing 2019 novel coronavirus S protein.

Auxiliary materials: mannitol, sucrose, sodium chloride, magnesium chloride, polysorbate 80, hydroxyethyl piperazine ethane sulfonic acid, glycerol

Specifications: 1.5ml/vial (15×10^{10}vp), stored according to the instructions.

Storage: storage and transportation at 2-8℃.

Inoculation route: oral atomization inhalation

Immunization procedure: use Continuous Vapouring System (manufactured by Suzhou Weiqi Biological Technology Co., LTD, Suzhou City, China) to aerosolized 0.1ml or 0.2ml of the Ad5-nCoV, and pour the droplets of vaccine into a disposable suction cup. Participants will inhale the aerosolized vaccine by mouth. Low dose aerosolized Ad5-nCoV group: use atomization device to atomize 0.1ml vaccine; High dose aerosolized Ad5-nCoV group: use atomization device to atomize 0.2ml vaccine.

4.2 Inactivated SARS-CoV-2 vaccine (Vero cells)

Manufacturer: Beijing Sinovac Research & Development Co., Ltd.

Specification: 0.5ml/ vial, for commercial packaging, storage according to the instructions.

EXP: please refer to the inspection report of National Institute for Food and Drug Control for details.

Storage: storage and transportation at 2-8℃.

Inoculated Pathway: intramuscular injection (IM) into the lower margin of the deltoid muscle of the lateral upper arm.

Immunization procedure: one dose of booster inoculation

5. Research Purposes

To evaluate safety and immunogenicity of a heterologous prime-boost immunization of Inactivated SARS-CoV-2 vaccine (Vero cells) and aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) in healthy adults aged 18 years and older.

6. Trial Design

This study is a single-center, randomized, open-label, parallel-controlled heterologous prime-boost immunization clinical trial.

6.1 Study Endpoints

6.1.1 Primary endpoints:

- GMT of neutralizing antibodies against live SARS-CoV-2 virus on day 14 after the booster vaccination.
- The incidence of adverse reactions in each group within 14 days after the booster

vaccination;

6.1.2 Secondary endpoints:

6.1.2.1 Safety endpoints

- Incidence of adverse events (AE) within 0-28 days after the booster vaccination;
- Incidence of serious adverse events (SAE) till the 12 months after booster; vaccination;

6.1.2.2 Humoral immunogenicity endpoints

- GMT, fold increase and seroconversion of neutralizing antibodies against live SARS-CoV-2 virus on day 7 and 28 after the booster dose in each group;
- Fold increase and seroconversion of neutralizing antibodies against live SARS-CoV-2 virus, as compared to baseline, on day 14 after the booster vaccination (≥4 times increased) in each group;
- GMT, fold increase and seroconversion of neutralizing antibodies against live SARS-CoV-2 virus at month 3, 6, and 12 after the booster dose in immunogenic subgroups;
- GMT, fold increase and seroconversion of binding antibodies against SARS-CoV-2 RBD on days 7, 14 and 28 after the booster dose in each group.
- GMT, fold increase and seroconversion of binding antibodies against SARS-CoV-2 RBD at month 3, 6, and 12 after the booster dose in immunogenic subgroups.

6.1.2.3 Cellular immunogenicity endpoints

- The levels of IFN-γ、IL-2 and IL-13 secreted by specific T cells on day 7 and 14 after the booster vaccination.

6.1.3 Exploratory endpoints:

- GMT, GMI and seroconversion positive conversion rate of anti-SARS-CoV-2 S protein SIgA antibody in saliva and IgA antibody in serum after the booster vaccination;
- GMT and GMI of neutralizing antibodies against SARS-CoV-2 in serum by anti-Ad5 adenovirus neutralizing antibody levels at 3, 6, and 12 months after the booster vaccination in the immunogenicity subgroups;
- Serum GMT of cross-neutralization test antibody levels to Delta VOCs in some participants at day 14 after the booster vaccination;
- GMT and fold increase of neutralizing antibodies against Ad5 at 3, 6, and 12 months after the booster vaccination in the immunogenicity subgroups;
- GMT of cross-neutralization antibody level in some participants at day 14 after the booster vaccination;
- Pulmonary function monitoring of the first 90 participants in the low-dose and high-dose aerosolized groups before immunization and at days 7, 14 and 28 after booster immunization (forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and peak expiratory flow rate (PEFR), etc.).

6.2 Sample size

(1) Hypothesis 1：GMT of group receiving heterologous boost with aerosolized Ad5-nCoV is not inferior to that in the group receiving homologous boost at Day 14 after the vaccination.

(2) Hypothesis 2: GMT of group receiving heterologous boost with aerosolized Ad5-nCoV is superior to that in the group receiving homologous boost at Day 14 after the vaccination.

*If hypothesis 1 is valid, further statistical inference is made for hypothesis 2. The above hypotheses are only for independent analysis.

Three to nine months after two doses of inactivated vaccine, the baseline GMT level before the booster immunization is expected to be about 1:40 ($\log_{10}X$=1.6). The GMT level after booster immunization with one dose of inactivated vaccine is estimated to reach 1:80 ($\log_{10}X$ =1.9). The GMT level after booster immunization with one dose of aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) is estimated to reach 1:160 ($\log_{10}X$ =2.2). The Standard Deviation is about 4 ($\log_{10}X$=0.6), the sample size is calculated:

For hypothesis 1, one-sided 2.5% significance level, 95% study power, GMT ratio of heterologous boost group/ homologous boost group non-inferiority margin is 0.67 ($\log_{10}X$ = -0.174), the ratio of heterologous boost group and homologous boost group is 1:1, and the sample size is calculated as 43 per group.

For hypothesis 2, one-sided 2.5% significance level, and the ratio of heterologous boost group and homologous boost group is 1:1. When it is proved that the GMT level of heterologous boost group is better than that of homologous boost group, under 95% study power, the sample size of each group is calculated to be about 105 per group. Considering a dropout rate of about 20%, the sample size is adjusted to 140 per group.

For safety evaluation, a sample size of 110 per group is estimated to provide a power of over 80% to detect an increase of 5% adverse reactions of heterologous boost group compared with that of the homologous boost group.

The study groups are shown in Table 1.

Table 1. Sample size of each study group

Study group	Vaccine / dose	Sample size	Interval after primary immunization
Low dose aerosolized Ad5-nCoV group	Aerosolized Ad5-nCoV/ 1.0×10^{10} vp (0.1ml)	140	3~9 months
High dose aerosolized Ad5-nCoV group	Aerosolized Ad5-nCoV/ 2.0×10^{10} vp (0.2ml)	140	3~9 months

Inactivated vaccine group	Inactivated COVID-19 vaccine /600SU (0.5ml)	140	3~9 months
Total		420	

Note: Participants recruited in must have complete 2 doses of inactivated vaccine immunization. The first 40 people in each group are selected to enter the expanded immunogenicity subgroup, to detect the response of cellular immune and the level of humoral immune persistence at 3, 6 and 12 months after booster immunization.

6.3 Study Plan

The project plans to enroll 420 healthy participants aged 18 and over.

Participants who had informed consent and passed the examination and screening were randomly assigned to low dose aerosolized Ad5-nCoV group, high dose aerosolized Ad5-nCoV group, and inactivated vaccine group. Participants recruited in need to complete the basic immunization with 2 doses of COVID-19 inactivated vaccine. After completing the basic immunization with 2 doses of inactivated COVID-19 vaccine, the enrolled participants will be boosted with 1 dose of inactivated COVID-19 vaccine or aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) in the 3 to 9 months.

All enrolled participants were collected venous blood to detect serum antibody level and were collected saliva for secretory IgA (sIgA) detection before the booster vaccination (day 0), and days 7, 14 and 28 after the booster vaccination. Among them, participants in the immunogenic subgroup need to be collected venous blood to detect serum antibody level and saliva for sIgA detection at 3, 6 and 12 months after the booster vaccination, and venous blood is collected to detect cellular immune indexes before the booster vaccination (day 0), day 7 and day 14 after the booster vaccination. For some participants, the cross-neutralization test needs to be conducted using their venous blood collected on the 14th day of immunization; about the first 90 participants in the low-dose and high-dose aerosolized groups need to undergo pulmonary function test on days 0, 7, 14 and 28. The three groups are enrolled in parallel. During the enrollment process, the safety data is evaluated in real time. Once safety problems were found in the vaccination and the suspension or termination criteria were met, the subsequent enrollment is immediately suspended or terminated.

This study needs to complete 7 visits, as follows:

V1: At day 0 (booster vaccination), informed consent and signing, physical examination, consultation screening, randomization, pulmonary function testing, pre-immunization blood and saliva collection, booster vaccination and observation are required;

V2: At day 7 after the booster vaccination, safety follow-up, pulmonary function test,

blood and saliva collection should be carried out;

V3: At day 14 after the booster vaccination, safety follow-up, pulmonary function test, blood and saliva collection should be carried out;

V4: At day 28 after the booster vaccination, safety follow-up, pulmonary function test, blood and saliva collection should be carried out;

V5: At month 3 after the booster vaccination, safety follow-up, blood and saliva collection should be carried out (only in immunogenic subgroup);

V6: At month 6 after the booster vaccination, safety follow-up, blood and saliva collection should be carried out (only in immunogenic subgroup);

V7: At month 12 after the booster vaccination, safety follow-up, blood and saliva collection should be carried out (only in immunogenic subgroup);

Table 2. Schedule and contents of visit plan for participants.

Visit No.	V1	V2	V3	V4	V5	V6	V7
Visit interval	Day 0	Day 7	Day 14	Day 28	Month 3	month 6	month 12
Time window		（+2 days）	（+3 days）	（+5 days）	（±15 days）	（±15 days）	（± 30 days）
Informed consent	●						
Demographic information collection	●						
Oral and nasal disease examination	●						
Axillary body temperature measurement	●						
Urine pregnancy test(for women of childbearing age only)	●						
Inclusion and exclusion criteria screening	●						
Randomization	●						
Pulmonary function testing	●	●	●	●			

Blood collection	●10ml (20ml)*	●10ml (20ml)*	●10ml (20ml)*	●10ml	●10ml*	●10ml*	●10ml*
Saliva collection	2ml	2ml	2ml	2ml	2ml*	2ml*	2ml*
Vaccination and Observation for 30 min post-vaccination	●						
Safety visit (AR/AE)	●	●	●	●			
Distribution diary card(within day 14)	●						
Return diary card (within day 14)and distribute diary card(14 days later)			●				
Return diary card (14 days later)				●			
Report serious adverse event (SAE)	●	●	●	●	●	●	●

* Indicates that it is only performed in the immunogenic subgroup

Note: Telephone follow-up/face-to-face interviews are conducted in the non-immunogenic subgroup at month 3, month 6 and month 12.

6.4 Randomization

In the study, eligible participants will be randomly assigned to low dose aerosolized Ad5-nCoV group, high dose aerosolized Ad5-nCoV group, and inactivated vaccine group. Authorized researchers can assign the study number to the enrolled participants through the IWRS system. Researchers in vaccine management use the corresponding group of study vaccines based on the research number and grouping information. The screening number is S + three digits (for example, S001, S002). The study number (random number) is V + three digits (for example, V001, V002).

6.5 Criteria for pausing or early termination

The principal investigator will organize an expert panel meeting to decide whether to terminate the clinical trial early, if one of the following situations occurs:

1) Occurrence of one or more grade 4 adverse event or serious adverse event that may be associated with vaccination;
2) Occurrence of grade 3 adverse events associated with vaccination in 10% of participants or more.

The study will be terminated in advance if one of the following situations occurs:
1) When the grade 4 adverse event or serious adverse event occurs that may probably be associated with vaccination, the principal investigator will organize a panel discussion to decide whether to terminate the study;
2) When the grade 3 adverse events associated with vaccination occur in 15% of participants or more and fail to be reduced to grade 1 or 2 for more than 48 hours, the principal investigator will organize a panel discussion to decide whether to terminate the study;
3) The principal investigator call for a complete termination of the trial and explain the reasons;
4) Ethics committee call for a complete termination of the trial and explain the reasons;
5) Administrative authority call for a complete termination of the trial and explain the reasons.

7. Participants

7.1 Participants selection

Healthy adults aged 18 years and older, who completed basic immunization of 2 doses of inactivated COVID-19 vaccine, are selected as participants with full informed consent.

7.2 Inclusion criteria

1) Health participants aged ≥18 years, completed two doses of inactive SARS-CoV-2 vaccine in the past 3-9 months.
2) The research participants can give informed consent and sign the informed consent form (ICF);
3) The participants are able and willing to comply with the requirements of the clinical trial program and can complete the 12-month follow-up of the study;
4) Axillary temperature ≤ 37.0 ℃.
5) No nasal or oral diseases, such as acute rhinitis (sinusitis), allergic rhinitis, oral ulcer, sore throat, etc.
6) Individuals who are in good health condition at the time of enrollment, which is determined by medical history, physical examination and clinical judgment of the investigator.

7.3 Exclusion Criteria

1) having a medical history or family history of convulsion, epilepsy, encephalopathy and psychosis;

2) suffering from abnormal pulmonary function such as asthma, chronic obstructive pulmonary disease and pulmonary fibrosis;
3) suffering from more serious cardiovascular diseases, such as arrhythmia, conduction block, myocardial infarction, severe and uncontrollable hypertension;
4) being allergic to any component of the research vaccines, or having a history of hypersensitivity or serious reactions to vaccination in the past;
5) women with positive urine pregnancy test, pregnant or breast-feeding, or have a pregnancy plan within twelve months;
6) have symptoms of upper respiratory tract infection;
7) having acute febrile diseases and infectious diseases;
8) have severe chronic diseases or condition in progress cannot be smoothly controlled, such as asthma, diabetes, thyroid disease;
9) congenital or acquired angioedema / neuroedema.
10) having the history of urticaria within 1 year before receiving the investigational vaccine.
11) having asplenia or functional asplenia.
12) having thrombocytopenia or other coagulation disorders (which may cause contraindications for intramuscular injection);
13) fainting during acupuncture (for inactivated vaccine group only).
14) having the history of immunosuppressive therapy (continuous oral administration or venous transfusion for more than 14 days), antiallergy therapy, cytotoxic therapy or inhaled corticosteroids over the past 6 months;
15) having received blood products within 4 months before injection of investigational vaccines;
16) having received another investigational product within 1 month before injection of investigational vaccine;
17) having received other attenuated live vaccine within 1 month before injection of investigational vaccine;
18) under anti tuberculosis treatment;
19) affecting the participants to sign the informed consent because of various medical, psychological, social or other conditions based on the judgment of the investigators.

7.4 Withdraw from the study

Participants have the right to withdraw from the study at any time during the study period, and the investigators should record the reason of withdraw:

(1) Loss of contact, early withdraw of the study;
(2) Request to withdraw without any reason;
(3) Withdraw for reasons unrelated to the study, such as long-term departure, relocation, etc., and the specific reason for withdrawal should be recorded;

(4) Withdrawal for reasons related to the study, such as intolerance of adverse reactions, intolerance of biological specimen collection, etc., and the specific reason for withdrawal should be recorded. If a participant withdraw because of AE or SAE, investigator should follow up the participant until the resolve of AE or SAE.

(5) Participants can require a complete withdraw from the study, all study behaviors can be stopped, including vaccination, biological specimen collection and safety observation. The data before withdrawal will not be used for analysis if he or she require so. If the participants allow the investigators use the data collected before the withdrawal, the data can be included in analysis. Otherwise not;

(6) Participants can require a partially withdraw from the study, such as refuse to vaccination or blood drawn only, but still participate in other procedures during the follow-up.

7.5 Complete of the study

7.5.1 Complete of the safety data collection

The participants who take the vaccination, and complete safety observation at day 28, and the occurrence of SAEs within 12 months after the last vaccination.

7.5.2 Complete of immunogenicity data collection

Blood samples and saliva are collected at day 0, day 7, 14 and 28 after immunization according to the protocol. Blood samples and saliva of participants are additionally collected at months 3, 6 and 12 in the immunogenic subgroup.

7.6 Definition and action taken of protocol violation and protocol deviation

7.6.1 Protocol violation (including but not limited to)

- No informed consent singed by the participant;
- The enrolled participant does not meet the all the inclusion criteria or meet one or more exclusion criteria;
- The participant received incorrect intervention;
- The participant received a vaccine fail to meet the requirements;
- Any other reasons identified by the investigators and confirmed by the principal investigator.

Any protocol violation should be recorded and reported to the principal investigator and the ethics review committee of the Jiangsu Provincial Center for Disease Control and Prevention.

7.6.2 Protocol deviation (including but not limited to)

- Beyond the visiting time window;
- Low compliance of participants, and the participants do not complete the blood sample collection;

- Serious adverse events do not report in time (SAE);
- Participants are treated with unallowed drugs (intramuscular, oral or intravenous corticosteroids for ≥2mg/kg/days, continuous use for ≥14 days, or other immunosuppressants);
- The interval between vaccination with other vaccines is insufficient;
- Other reasons considered as protocol deviation by the principal investigator.

Any protocol deviation should also be recorded and reported to the principal investigator.

8. Methods and procedures

8.1 Participants selection

Recruitment for clinical trials will be conducted at the research site. Recruitment targets are healthy adults aged over or equal to 18 years whom have received two doses of inactivated SARS-CoV-2 vaccines. Recruitment of participants is carried out by researchers with work experience.

8.2 Informed consent

When obtaining and recording informed consent, researchers should abide by relevant regulations, GCP and the ethical principles stipulated in the Declaration of Helsinki. Before the start of the study, the investigator should obtain written approval/consent from the ethics review committee for the informed consent form and other documents provided to the subject.

Before participating in this clinical study, researchers should explain the contents of the informed consent form to the participants and/or their witnesses, and the participants and/or their witnesses should be given sufficient time to consult the details of the study before signing the informed consent form. When explaining the information of informed consent to multiple persons, each subject and/or witness should be given the opportunity to ask the investigator individually before signing the informed consent form.

Researchers should keep the informed consent form signed by each subject, and provide the subject with a copy of the signed name and date of the informed consent form.

8.3 Research site registration

After the participants sign the informed consent form, the researcher assigns the screening ID number according to the order of arrival. The screening ID number is S+three-digit number, and the number range starts from S001.

8.4 Physical examination and screening

Before enrollment, participants are required to take axillary temperature measurement, and oral and nasal cavity examinations. Urine pregnancy tests will be performed on women of childbearing age.

According to the “inclusion and exclusion criteria”, the interviewers conduct

medical history inquiry and screening for participants. Only those who passed the screening can be enrolled and participate in the randomization.

8.5 Study groups

After the participants pass the examination, they will be randomized. Authorized researchers log in to the IWRS system, and assign research numbers to the enrolled participants. The vaccine management researchers use the corresponding group of research vaccines based on the research number and grouping information.

Participants who fall off without being vaccinated after randomization can be substituted by other eligible participants. For example, if the V001 subject withdraw without being vaccinated, the next subject to be enrolled will be a substitute subject, whose random number is V1001. If the substitute subject also withdraws without being vaccinated, the next subject to be enrolled will be another substitute subject, whose random number is V2001. There are up to 9 substitute participants for 1 participant.

8.6 Vaccine distribution and inoculation

The subject information should be checked before vaccination, and the vaccination can be performed only if the subject meets inclusion criteria of this clinical research protocol.

First aid drugs such as epinephrine hydrochloride and first aid equipment such as simple ventilator and ECG monitor should be provided at the vaccination site.

8.6.1 Immune route and immune program

The booster immunization schedule: Participants are additively vaccinated with 1 dose of aerosolized Ad5-nCoV or inactivated SARS-CoV-2 vaccine in 3 ~ 9 months after the completion of 2 doses of inactivated SARS-CoV-2 vaccine. The vaccine should be shaken thoroughly before use and used immediately after opening. In case of cracks, unclear label, failure, or abnormal appearance of the vaccine, it should not be used.

The inactivated SARS-CoV-2 vaccine is injected into the outer deltoid muscle of the upper arm for immunization, and the aerosolized Ad5-nCoV is immunized by oral aerosol inhalation.

1) Intramuscular injection:

The injection site is disinfected with 75% alcohol before injection, and the inactivated SARS-CoV-2 vaccine is injected intramuscularly after the injection site is slightly dry. The vaccine should be fully shaken before use. The vaccine cannot be injected intravenously, intradermally or subcutaneously. After vaccination, the participants should be carefully observed for at least 30 minutes, and appropriately medical treatment should be prepared to deal with the possible allergic reaction after vaccination.

2) Aerosol inhalation through Continuous Vapouring System: See SOP for details.

Notes for attention:

(1) Vaccine should be added into the bottom of atomizing cup of the Continuous Vapouring System to avoid splashing on the wall of the atomizing cup , and the vaccine syringe needle should not touch the bottom of the atomizing cup .
(2) All participants do not need to clean their mouth before inhalation, and they need to abstain from water and food for half an hour after vaccination.
(3) The atomizer of the Continuous Vapouring System can be reused, but the atomizing cup can only be used for a single time. The atomizer will be handed over to investigator at the end of the day of immunization.
(4) Keep the atomizing cup upright during atomization, and keep it level during inhalation. Handle the atomizing cup gently, do not shake or press.

Participants inhale the aerosolized Ad5-nCoV through deep breathing. After inhalation, they need to hold their breath for 5 seconds before exhaling.

8.6.2 Management of vaccines

1) Vaccine storage：The temperature of vaccine storage place should be controlled in the range of 2-8 ℃ to prevent freezing; the storage temperature of vaccine should be recorded once respectively in the morning and afternoon of each working day. The time of everyday temperature record should be the same as possible。

2) Vaccine transportation：The partner is responsible for transporting the research vaccines from vaccine manufacturers to the clinical research site. The responsible institution (Jiangsu Provincial Center for Disease Control and Prevention (Jiangsu Public Health Research Institute) and the vaccine management personnel of the research site will jointly check the temperature record during transport, the inspection report (passing) and the package of the vaccine, and finally sign together for receipt if there is no problem.

8.7 Safety follow up and evaluation

8.7.1 Safety observation

After vaccination, the participants will stay at the clinic for 30-minutes safety observation. The trained researchers should systematically observe each subject, and record the local and systemic reactions and their severity within 30 minutes.

Within 14 days after vaccination, the participants are urged to complete the safety observation by themselves, and record the safety data on the "Diary Card (within 14 days)". At day 7 and day 14, the investigator will visit the participants and conduct a retrospective investigation to verify the safety observation content.

From day 15 to day 28 after vaccination, the participants will complete the safety observation on their own and record the observation results on the "Diary Card (after 14 days)". At day 28, the investigator will visit the participants, conduct a retrospective investigation to verify the safety observation content. During the study period (within 12 months after vaccination), serious adverse events will be collected through the active report of subject and the regular follow-up of investigator.

About the first 90 participants in the low-dose and high-dose aerosolized Ad5-nCoV groups need to undergo pulmonary function examination before vaccination and at day 7, day 14 and day 28 after vaccination. The items include forced vital capacity, vital capacity in the first second, maximum expiratory flow rate, etc. The result of pulmonary function test will not be carried out the correlation judgment and severity classification.

8.7.2 Safety observation contents and indicators

Based on the types and incidence of adverse reactions observed in the previous phase I, II, and III clinical trials of the research vaccines and referring to the adverse reactions listed in the instructions of similar products on the market, the injection site pain, induration, swelling, rash, redness, itch and cellulitis are listed as the solicited AEs at injection site after intramuscular injection, the xerostomia, hoarseness, oral mucositis and garget are listed as the solicited local AEs after aerosol inhalation, and the diarrhea, nausea, vomiting, anorexia, fatigue, headache, arthralgia, muscle pain at non- injection site, itching at non- injection site (no skin damage), skin and mucous membrane abnormalities, pharynx pain, cough, chest pain, runny nose, and sneezing are listed as the solicited non-injection site (systemic) AEs. The other AEs are non-solicited. AEs are graded according to the guiding principles for the classification of adverse events in clinical trials of preventive vaccines (NMPA [2019] No. 102), as follows: (table 3-5)

Table 3. Grading of (local) AEs at injection site

Symptoms /Signs	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Pain	Do not affect or slightly affect physical activity	affect physical activity	Affect daily life	Loss of basic self-care ability or hospitalization
Induration*, swelling (optional)** #				
>14 years	Diameter 2.5~＜5 cm or area 6.25~＜25 cm^2 and does not affect or slightly affect daily life	Diameter 5~＜10 cm or area 25~＜100 cm^2 or affect daily life	Diameter ≥ 10 cm or area ≥ 100 cm^2 or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or seriously affect daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Rash*, Redness (optional)** #				

>14 years	Diameter 2.5~＜5 cm or area 6.25~25 cm^2 and does not affect or slightly affect daily life	Diameter 5~＜10 cm or area 25~＜100 cm^2 or affect daily life	Diameter ≥ 10 cm or area ≥ 100 cm^2 or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or seriously affect daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Others				
Itch	Itching at the vaccination site, relieved by itself or within 48 hours after treatment	Itching at the vaccination site, which does not resolve within 48 hours after treatment	Affect daily life	NA
Cellulitis	NA	Non-injectable treatment is required (e.g. oral antibacterial, antifungal, antiviral therapy)	Intravenous treatment is required (e.g. intravenous antibacterial, antifungal, antiviral therapy)	Sepsis, or tissue necrosis, etc.
Dry mouth (Xerostomia)	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA
Hoarseness	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA
Oral mucositis	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA

Garget	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA

Note: *in addition to directly measuring the diameter for grading and evaluation, the progress of the measurement results should also be recorded.

** the maximum measuring diameter or area should be used.

the evaluation and grading of induration and swelling, rash and redness should be based on the functional level and the actual measurement results, and the indicators with higher classification should be selected.

Table **4.** Grading of (systemic) adverse events.

Symptoms /Signs	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Diarrhea	Mild or transient, 3 to 4 times a day, abnormal stool, or mild diarrhea last less than 1 week	Moderate or persistent, 5-7 times a day, abnormal stool characteristics, or diarrhea ＞1 week	＞7 times/day, abnormal stool, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, need intravenous infusion ＞2L	Hypotension shock, hospitalization required
Constipation*	Requires stool softener and dietary modification	Need for laxatives	Stubborn constipation requires manual dredging or the use of enemas	Toxic megacolon or ileus
Non-injection-site muscle pain	Does not affect daily activities	Slightly affect daily activities	Severe muscle pain that seriously affects daily activities	Emergency or hospitalization

Arthritis	Mild pain with inflammation, erythema, or swelling of joints; but does not interfere with function	Moderate pain with inflammation, erythema, or swelling of joints; impairs function but does not affect daily activities	Severe pain with inflammation, erythema, or joint swelling; affecting daily activities	Permanent and/or disabling joint injury
Arthralgia	Mild pain without hindering function	Moderate pain; need analgesics and/or pain that impedes function but does not affect daily activities	Severe pain; need analgesics and/or pain affecting daily activities	Disability pain
Pectoralgia	Mild pain without hindering function	Moderate pain; need analgesics and/or pain that impedes function but does not affect daily activities	Severe pain; need analgesics and/or pain affecting daily activities	Disability pain
Headache	Does not affect daily activities and requires no treatment	Transient, slightly affects daily activities and may require treatment or intervention	Seriously affects daily activities and requires treatment or intervention	Intractable and requires emergency or hospitalization

Syncope	Close to syncope without losing consciousness (pre-syncope)	Loss of consciousness without treatment	Loss of consciousness and needs treatment or hospitalization	NA
Convulsions				
≥18 years	NA	NA	1～3 times convulsions	convulsions (e.g. status convulsion) or difficult to control (e.g. intractable epilepsy)
Non-injection-site itching (no skin lesions)	Slightly itchy without affecting or slightly affecting daily life	Itching affects daily life	Itching makes it impossible to carry on daily life.	NA
Abnormal skin and mucosa	Erythema/itching/color change	Diffuse rash/macular papule/dryness/desquamation	Blister/exudation/desquamation/ulcer	Exfoliative dermatitis involving mucous membrane, or erythema multiforme, or suspected Stevens-Johnsons syndrome

Insomnia*	Mild difficulty in falling asleep, not affecting or slightly affecting daily life	Moderate difficulty in falling asleep, affecting daily life	Serious difficulty in falling asleep, seriously affecting daily life, requiring treatment or hospitalization	NA
Irritation or depression	Mild irritability or mild depression	Irritability or somnolence	Unable to soothe or become hyporesponsive	NA
Mental disorder (includes anxiety, depression, mania, and insanity) Detailed symptoms to be reported	Minor symptoms that do not require medical attention or behavior do not influence or slightly influence daily life	Clinical symptoms requiring medical attention or behavior influencing on daily life	Requires hospitalization or inability to perform daily life	Have a tendency to hurt yourself or others or acute insanity or loss of basic self-care ability
The immune system				
Acute allergic reaction **	Local urticaria (blister) without treatment	Local urticaria requiring treatment or mild angioedema without treatment	Extensive urticaria or angioedema requiring treatment or mild bronchospasm	Anaphylactic shock or life-threatening bronchospasm or throat edema
Fatigue	Does not affect daily activities	Affects normal daily activities	Seriously affects daily activities and cannot work	Emergency or hospitalization

Non-injection-site pain# (Specify the location when reporting)	Minor pain that does not affect or slightly affect daily life	Pain affects daily life	Pain can't carry on daily life	Disability pain, loss of basic self-care ability
Dysphagia	Mild discomfort when swallowing	Diet is restricted	Diet and conversation are very limited; you can't eat solid food.	Can't eat liquid food; need parenteral nutrition.
Anorexia	Loss of appetite, but no reduction in food intake	Loss of appetite, reduced food intake, but no significant weight loss.	Loss of appetite and weight loss	Need for intervention (e.g. gastric tube feeding, parenteral nutrition)
Vomiting	1- 2 times/24 hours and does not affect the activity	3- 5 times/24 hours or activity is restricted	＞6 times/24 hours or need intravenous rehydration	Hypotension shock requires hospitalization or other means of nutrition
Nausea	Transient (<24 hours) or intermittent and food intake is normal	Continued nausea leads to reduced food intake (24-48 hours)	Persistent nausea results in almost no food intake (> 48 hours) or requires intravenous fluid replacement	Life-threatening (eg hypotension shock)

Cough	Transient, without treatment	Persistent cough, effective treatment	Paroxysmal cough, uncontrollable treatment	Emergency or hospitalization
Runny nose	Transient, without treatment	Persistent runny nose, effective treatment	Uncontrollable treatment	Emergency or hospitalization
Sneezing	Transient, without treatment	Persistent sneezing, effective treatment	Uncontrollable treatment	Emergency or hospitalization
Acute bronchospasm	Transient; no treatment required; FEV_1% 70-80%	Requires treatment; bronchodilator therapy normalized; FEV_1% 50-70%	Bronchodilator treatment does not return to normal; FEV_1% 25% to 50% or persistent depression in the intercostal space	Cyanosis; FEV_1% < 25%; or intubation required
Dyspnea	Dyspnea on exercise	Dyspnea with normal activity	Dyspnea at rest	Dyspnea requiring oxygen therapy, hospitalization or assisted breathing
Sore throat***	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe in severity, severe interference with daily activities, treatment required	NA

Note: FEV_1% refers to forced expiratory volume in the first second (FEV_1)/forced vital capacity (FVC)

* For constipation and insomnia, attention should be paid to changes before and after vaccination

** refers to type I hypersensitivity.

Refers to Non-injection-site pain other than muscle pain, Arthralgia and headache.
*** Refer to《Guidelines for grading standards of adverse events in clinical studies of prophylactic vaccines》

Table 5 Vital Signs Grading Scale

Signs	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Fever * [Axillary temperature (℃)]				
＞14 years	37.3～＜38.0	38.0～＜38.5	38.5～＜39.5	≥39.5, for more than 3 days

Note: * Axillary temperature is generally used in China. It can be converted in oral temperature and rectal temperature if necessary. Oral temperature =Axillary temperature +0.2℃; Rectal temperature = Axillary temperature ＋（0.3~0.5℃）.When persistent high fever occurs, the cause of high fever should be identified as soon as possible.

For adverse events that do not reach level 1 in the above table, it can be recorded as level 0.

General principles for the grading for other adverse events

The intensity of adverse events not mentioned in the rating table shall be evaluated according to the following criteria:

Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Mild: Short-term (< 48 hours) or mild discomfort, no influence on activities, treatment not indicated	Moderate: Mild or moderate restricted activities, presentation indicated possibly, treatment not indicated or mild treatment indicated	Severe: Significant restricted activities, presentation and treatment indicated, hospitalization indicated possibly	Critical: Life-threatening possibly, severely restricted activities, intensive care indicated	Death

8.7.3 Outcome of AEs

The outcomes of ARs/AEs include: (1) Recovery; (2) Not yet recovered; (3) Recovered with sequelae; (4) Death; (5) Loss of visit.

8.7.4 Relationship between AE and vaccination

Investigators should try their best to judge AE and evaluate its possible causal

relationship, that is, the causal relationship between vaccination and alternative causes (such as history of underlying diseases, combined treatment). This applies to all AEs, including serious ones and non-serious ones.

Causality assessment will be determined by the extent to which an event can be reasonably explained in one or more of the following aspects:

- Similar reactions are observed in the past;

- For similar types of vaccine, the same events have been reported in the literature;

-The adverse event occurs at the time of the first vaccination and occurs again at the time of the re-vaccination.

The causal relationship of AE should be evaluated by the investigator according to the following aspects.

-Related: it is suspected that there is a connection between the study vaccine and the AE (it is not necessary to determine the degree of possibility); The study vaccine has a reasonable possibility to induce the AE.

-Unrelated: there is no connection between the study vaccine and the AE; There are other more likely causes, and the study vaccination is not suspected to induce the AE.

8.7.5 Treatment of SAEs/SARs

Adverse event (AE) is any untoward medical occurrence in a patient or clinical trial participant administered with a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

Adverse reactions (AR): unexpected or harmful reactions in the process of vaccination according to the prescribed dose and procedure, usually related to vaccination.

Serious adverse event (SAE): refers to the following important medical events, whether or not related to the vaccine clinical trial, including: 1) death; 2) life threatening; 3) hospitalization or prolonged hospitalization; 4) permanent or significant disability / loss of function; 5) congenital abnormality or birth defect; 6) other important medical events, which without treatment may lead to those events listed above.

Suspected Unexpected Serious Adverse Reaction (SUSAR): refer to the adverse reactions of participants at any dose that have nothing to do with the purpose of the medication. After analysis, it is considered that the relationship of the adverse reactions with the drug is at least likely to be related. The nature, extent, consequences, or frequency of the unexpected adverse reaction are different from the expected risks described in the previous plan or other related materials (such as the investigator's manual and instructions).

The participants should report to the investigator as soon as possible if there is any clinically significant disease/event after vaccination. The investigator should follow up the adverse reaction/event until the symptoms disappear or the symptoms stabilize.

When necessary, the appropriate treatment will be provided unconditionally to relieve the pain caused by the adverse reaction/event for the subject. All medical treatment will be recorded at each follow-up.

Once a serious adverse event occurs, the investigators should deal with it quickly, fill in the "Serious Adverse Event Report Form" within 24 hours, and report it to the principal investigator in the form of fax or E-mail.

8.7.6 Reporting procedures for SAE

Any SAE, whether related to the research vaccines or not, must be reported to the principal investigator via fax or E-mail within 24 hours of being informed. The report information includes description of the SAE, timing and type of onset, duration, intensity, causality with vaccination, outcomes, management (symptomatic treatment), and other relevant clinical and laboratory data.

Once upon receiving the report of a SAE, the principal investigator and the safety observer need to decide the participant who has the SAE whether continues to participate in the study or not, based on the duration, extent, intensity and outcome of the SAE and the willingness of the participant. The principal investigator should determine whether the SAE is SUSAR or not. If so, the principal investigator should report to the vaccine manufacturer, the Drug Evaluation Center of the National Medical Products Administration and the National Health Commission in time.

For SUSAR that is fatal or life-threatening, the principal investigator should report to the Drug Evaluation Center of the National Medical Products Administration as soon as possible after first being informed, but within 7 natural days, and within the following 8 days to improve the follow-up information.

Note: the day when the principal investigator is first informed the SUSAR is day 0.

For SUSAR that is not fatal or life-threatening, or other potentially serious security risks, the principal investigator should report to the Drug Evaluation Center of the National Medical Products Administration as soon as possible after first receiving the information, but within 15 natural days.

SAE should be truthfully recorded, evaluated and discussed in the final report after completion or termination of the trial.

8.7.7 Clinical assessment

The investigators should report any AE with clinical manifestations as soon as possible, and timely conduct the investigation and medical visit, such as medical history record, physical examination and necessary laboratory examination, as well as appropriate medical treatment. Follow-up of SAR/SAE should be continued until the SAE is resolved. The detailed investigation and follow-up record of SAR/SAE should be carried out, which context includes the following:

1) Description of AE;

2) Start and end time of AE;

3) Strength grading;

4) Association with vaccination;

5) Laboratory test results;

6) Treatment measures.

8.7.8 Pregnancy events

Before vaccination, the female participants will be given urine pregnancy test, and those who are positive in the urine pregnancy test should not be enrolled in the trial. If pregnancy event occurred within 12 months after vaccination, the investigator should fill in the Pregnancy Event Report Form.

8.7.9 Combined medication/vaccine

When the medical events happen during the study period, the participants are allowed to carry out the appropriate medical treatment, and the medical treatment should be recorded in time.

Other vaccination is not recommended except for emergent vaccination during the research period, such as rabies vaccine, tetanus vaccine, or other emergent use of vaccines. Any vaccine received is required to be recorded during the study period.

8.8 Collection, preservation and transportation of samples

8.8.1 Samples collection

All participants will be collected 10ml of venous blood with evacuated and promoting coagulating tubes before the booster immunization and on days 7, 14 and 28 after the booster vaccination. The serum separated from venous blood will be used to detect the level of antibodies induced by the vaccine. 10ml of venous blood from participants in the immunogenic subgroup needs to be collected before the booster vaccination and on days 7 and 14 after the booster vaccination, and then will be used for the detection of cellular immune indicators. 2 ml saliva of all participants need to be collected for SIgA detection before immunization and at days 7, 14 and 28 after the booster vaccination. 10ml venous blood and 2ml saliva from participant in the immunogenic subgroup also need to be collected in months 3, 6 and 12 after the booster vaccination for the detection of serum antibody and saliva SIgA respectively.

Blood samples from this clinical trial will be used to test the immune response indicators specified in the protocol, and their use for other studies need to be previously approved by the ethics committee.

8.8.2 Preservation and transportation of samples

Serum separation is performed in accordance with standard operating procedures, and the storage temperature of serum should be - 20℃ and below. The separation, transportation and preservation of PBMC used for the detection of cellular immune indexes are operated by the third-party laboratory according to the standard operating procedures.

Saliva samples should be stored at -20℃ and below and be transported to the testing laboratory in time.

9. Data administration

9.1 Data administration

In this study, EDC is used to collect and manage research data, and the system keeps a complete modification track to ensure the traceability of clinical trial data. According to the requirements of Technical Guidelines for Clinical Trial Data Management, data management includes data collection, input, cleaning, consistency check, database locking and other work. The data management process should comply with the GCP to ensure the authenticity, integrity and accuracy of clinical trial data.

9.1.1 Design and establishment of database

The project database (eCRF) is set up by the database designer, using the CDISC standard as much as possible.

After the database is established and tested, authorized PI, PM, CRA, DM, etc. can officially launch and apply the database after training.

9.1.2 Data entry

The trained personnel for data entry shall complete the data entry online in time after each visit.

The researcher needs to approve the data on eCRF to verify that the data recorded in eCRF is true. After data entry is completed, any data changes need to be explained (Comments) and automatically recorded in the system.

9.1.3 Verification of data records

The quality control personnel shall check the data records entered into the EDC regularly or irregularly to ensure that all the data entered are consistent with the original documents. If there are inconsistencies, quality control personnel need to send queries about the inconsistencies to the researcher in the EDC system, and the researcher needs to verify the original data and update the input content. Before locking the database, quality control personnel need to carefully verify the original data and the necessary signature of the researcher.

9.1.4 Verification of data

The data manager will question and manage the test data according to the Data Verification Plan (DVP).

When data is inputted into the EDC system and there is any illogical data, the system will automatically check and query. These queries need to be reviewed and answered by the researcher or authorized personnel. When the updated data makes the logical check impossible, the query will automatically shut down. DM can review the query which is automatically closed. If the query is not solved, DM can manually add questions, and continue to communicate with the research center until the query is solved.

In addition to automatic verification by the system, query can be manually added to the EDC system when there are questions checked out by SAS programming or the data administrator. Researchers need to clarify the manually added query.

Before locking the database, the data administrator must ensure that all the queries are cleaned up and the researcher has completed the electronic signature on the EDC system, and ensure the integrity and accuracy of clinical trial data.

9.1.5 Medical coding

Medical coders do the work of medical coding. AE will be coded according to the MEDDRA (version 23.1 or above) dictionary.

During the coding process, DM can communicate with the researcher in real time online for any encoding failure due to improper, inaccurate or ambiguous medical terms.

A medical review of the medical code is required before database locking.

9.1.6 Database locking

Complete the list of database locking. According to the procedure of database locking, the data manager, statistician and principal investigator need to sign the written approval of database locking. The data manager then export data from the database in a specified format and hand it over to the statistician for statistical analysis. After database locking, if there is conclusive evidence that it is necessary to unlock the database, the researcher and relevant personnel should sign the unlock document.

9.1.7 Outboard data manager

Immunogenicity data is managed as external data and needs to be reviewed and verified by data manager.

9.2 Statistical Analysis

9.2.1 Selection of analysis data sets

Safety set (SS):

The safety evaluation should be conducted for all participants who receive vaccines after randomization. Data of the protocol violation should not be eliminated.

Immunogenicity data set:

Full Analysis Set (FAS): It is defined as ideal participant population determined according to the ITT (Intention-to-treat analysis) principle, all participants who meet the inclusion / exclusion criteria, are randomized and given vaccines and have at least one test of immunogenicity are included in the FAS.

Per-Protocol Set (PPS): It is a subset of FAS. Participants in this set are more compliant with the protocol, experience no major protocol violation, comply with all inclusion criteria / exclusion criteria, complete the vaccination within the time window as required in the protocol and complete all blood samplings are included in the PPS set. Participants who violate the trial protocol, such as poor compliance or lost to follow-up, and those who suffer intercurrent SARS-CoV-2 infection will not be included in this analysis set.

In this trial, the FAS will be used as the primary analysis set. However, PPS should

be analyzed simultaneously. Any inconsistency between PPS and FAS analysis results should be discussed in the report.

9.2.2 Data statistics method

During statistical analysis, first, the number of completed cases and drop-out cases should be checked. Then demographic and baseline characteristics of each group at enrollment should be analyzed to investigate intergroup comparability. Efficacy evaluation of vaccine includes the determination of evaluation indicators and intergroup comparison of efficacy. Safety evaluation includes the statistics of clinical ARs/AEs.

Participant elimination criteria: participants don't meet the inclusion criteria; data and information after vaccination are not followed up; information and data after randomization are seriously missing; participants meet exclusion criteria but are not withdrawn; participants receive wrong vaccination or incorrect dose.

Safety analysis in this trial mainly includes descriptive analysis of the incidence of ARs/AEs. χ^2 test may be carried out for intergroup comparison, and Fisher's exact test may be performed if necessary. After immunization, the number of case-times and person-times of AEs will be calculated (with conventional calculation method). The number of person-times will be calculated based on the highest severity, and the number of case-times will be calculated based on the cumulative AEs. Logarithmic transformation is required for analysis of immunogenicity indicator of antibody level which should be expressed as GMT, standard deviation, median, maximum and minimum and 95% confidence interval. Classification indicators will be compared between groups. Antibody seroconversion rate will be analyzed by χ^2 test and Fisher's exact test may be used if necessary. Study data at different time points will be analyzed through statistical analysis for repeated measurement data.

SAS 9.4 is adopted for all statistical analyses with two-sided test. The *P* value is directly calculated while carrying out Fisher's exact test when test statistics and corresponding *P* values are given, $P \leq 0.05$ is viewed as statistically significant.

9.2.3 Statistical analysis plan

1) The safety data and immunogenicity data of the 28th day after the completion of the immunization program shall be analyzed for the first time after the results are verified to be correct.

2) The safety data and immunogenicity data of the 12 months after the completion of the immunization program shall be analyzed for the second time after the results are verified to be correct.

3) The first analysis does not consider consumption α.

9.2.4 Analysis software

All tests are analyzed by SAS software 9.4 or above version.

10. Monitoring of clinical trial

10.1 Quality assurance and quality control

Carrying out on-site quality control in strict accordance with the relevant requirements of Good Clinical Practice (GCP).

Investigators in some positions are qualified as physicians or above. Prior to the

clinical trial, they will be trained in the clinical protocol and all trial procedures, including information about the investigatonal vaccine, procedures for obtaining informed consent, operating procedures for each position, and procedures for reporting adverse reactions/events.

The data of each subject is reviewed at each stage of the clinical trial to ensure that the content of the clinical trial meets the requirements of the protocol and that the obtained data are complete and reliable. The quality controller controls the whole process of the clinical trial.

All the works on site are carried out strictly in accordance with the clinical trial field operation manual. Each subject records the "*Diary Card*" by themselves, follows up and retrospectively investigations by the researcher, and reviews and guides the filling in of the "*Diary Card*".

The quality controller shall conduct a comprehensive check on the original data, and after training, a assigned person shall enter the data of eCRF.

Calibration or standardization of the instruments used in this clinical trial is performed.

10.2 Modification of clinical protocol

After this plan is approved by the Ethics Committee, if there is any major modification in the implementation process, it shall be reported to the Ethics Committee for approval before it can be implemented. The Investigator shall not execute any deviation or change without the consent of the Sponsor and prior review and written approval of the Ethics Committee (EC).

Any changes to the scheme, whether material or non-material, are required to be in writing. EC approval is required to identify substantive protocol changes that would affect the safety of participants, the scope of the study, or the scientific quality of the study.

10.3 Scheme deviation

The investigator shall carry out the clinical trial according to protocol approved by the ethics committee and the provisions of GCP. During the trial, the researcher shall not deviate from the protocol unless the harm to the participants is eliminated.

The research center shall record all protocol deviations in the original data of participants, including but not limited to the occurrence time of protocol deviation, discovery time, event description and measures, etc. In case of serious protocol deviations, the main researchers should be informed in time and report to the IEC.

10.4 Confidentiality

The investigators, IEC, or a fully authorized representative of regulatory authority should have the right to obtain data related to the clinical trial, but relevant content cannot be used for any other clinical trials, nor can it be disclosed to any other individuals or entities.

Investigators must sign a confidentiality agreement to confirm that he/she knows and agrees to hold the information of this study confidential.

Investigators and other study personnel should keep all information provided by the Sponsor and all data/information generated at the study site (except for medical records of participants) confidential. Such information and data should not be used for any purposes other than the study. This restriction does not apply to: (1) study information is not disclosed because of violations by investigators and researchers; (2) study information is disclosed only to the IRB/IEC for the purpose of study evaluation; (3) study information is disclosed to provide appropriate medical assistance to participants.

10.5 Quality control of documents

10.5.1 Raw data

1) Vaccination and visit records;
2) Informed consent;
3) Sample collection records;
4) Vaccine immunization records;
5) Observation results and records of adverse events;
6) Cold chain records;
7) Records of vaccine handover, use, distribution and recycling.

10.6 Quality control of biological sample

10.6.1 Quality control of biological sample collection

Sampling personnel should verify the basic information and procedures in the original record book, local disinfection of blood collection should be conducted before blood collection, and subject ID and specimen number must be marked in relevant documents and collection vessels after specimen collection, and correct check should be made on the spot. After blood collection and numbering, the sampler should sign on the corresponding position in the original record book. Unusual conditions of blood collection should be accurately recorded.

A assigned person is responsible for the quality inspection of blood collection process, specimen quality and document filling. In case of wrong number, duplicate number and unqualified specimen, the person in charge of the site should get in touch immediately and remedy in time.

The collected samples should be properly kept and handed over to the blood sorting staff in the laboratory in time with a record of handover. Medical waste should be classified and placed according to the requirements and handed over to the relevant person in charge in time.

10.6.2 Quality control during the transportation of biological sample

On-site specimens will be transported to the specimen testing laboratory and all sample shall be recorded in accordance with the site standard operating regulations.

The site logistics manager shall sort out the specimen transport list before sending

the sample: the contents shall include the sample number, sample box number, sample number, etc. The paper copy of the sample transport list will be shipped with the sample.

Upon receipt of specimens from the laboratory, the recipient should check the number and condition of the sample, check whether the sample are consistent with the waybill, check whether the sample number is unique, and sign the specimen transport bill.

Temperature monitoring records should be recorded during the transportation of serum sample.

10.6.3 Quality control of biological sample preservation

The temperature of all refrigerators related to the project should be monitored twice in the morning and in the evening. If the temperature is abnormal, the person in charge of the refrigerator should fill in the cause of the abnormality and the treatment measures on the temperature monitoring form. Including cold chain interrupt alarm and other processing.

11. Risk management plan

11.1 Safety specifications

Including significant identified risks, significant potential risks, and significant missing information. According to the past clinical study summary of ADR, drug pharmacokinetics characteristics of products, risk of medical treatment/intervention, same class effect, indications, the epidemiological characteristics of the target population, the safety risks observed in non-clinical trials (including toxicology, drug interactions, etc.), and the population not studied in clinical trials are comprehensively considered.

11.2 Pharmacovigilance plan

Closely monitor and report the SUSAR and potential safety risks of the studied vaccines with reference to domestic and foreign research data, literature or reports related to the safety of similar vaccines; If major safety risks have been warned or reported, a risk control plan shall be developed and necessary measures shall be taken to protect the safety of participants. Safety monitoring data in clinical trials should be regularly summarized and analyzed in accordance with relevant requirements. In the analysis of monitoring data, the safety risk signals of drugs are focused. Based on the analysis of the monitoring data, the differences between the monitoring data and the safety information of the drug instructions are further identified, the occurrence of new and serious AR is analyzed, the need for risk management measures is discussed, and the opinions of benefit risk assessment are put forward.

11.3 Risk minimization measures

The main measures include: timely updating and revising the inclusion criteria and informed consent of the research protocol according to the information collected from long-term observation, follow-up and follow-up; Additional risk minimization

measures include risk classification of identified risks and recommendations for treatment, enhanced communication with participants, and training of participants to convey relevant treatment recommendations for risk classification, etc. In the trial scheme, unified safety evaluation standards and methods are formulated according to the corresponding guidelines issued by the national bureau, and the safety of the vaccine is actively monitored and followed up.

12. Schedules

Total study time: The clinical trial is planned to last about 14 months.

13. Ethical Approval

The clinical trial protocol shall be approved by the IEC. The principal investigator submits the clinical plan and all necessary additional documents to the IEC. After the approved, the Ethics Review Approval Document will be issued to the investigator.

At the same time, the researcher needs to provide a sample of informed consent to the IEC, which will examination and approval it.

Before signing the informed consent, participants have sufficient time to consider whether or not to participate in the study. Participants will have the opportunity to ask about the details of the trial and receive detailed answers. During the trial, the participants have the right to decide whether to withdraw from the trial

13.1 Ethical Review and Approval

The PI should submit the clinical trial protocol and all necessary additional documents to the IEC for initial review:

- Clinical study protocol (indicated with version No. / date)
- Informed Consent Form (indicated with version No./date)
- Participant recruitment materials (indicated with version No. / date)
- Diary card (indicated with version No. / date)
- Contact card (indicated with version No. / date)
- Vaccination Visit Record (indicated with version No./date)
- PI's CV

13.2 Supervise the following processes

13.2.1 Informed consent

Whether methods of selection of participants and provision of relevant information to participants are complete and easy to understand; Whether the method of obtaining informed consent is appropriate. Throughout the trial, the IEC will monitor whether there are ethical issues that harm the participants and whether they are treated or compensated for the harm caused by the trial, as well as assess the extent to which the participants are exposed to risk.

13.2.2 Confidentiality

Ensuring the personal confidentiality of the participants under the conditions of experimentation, biological sample collection, reporting, as well as publication. Only the ID number are recorded for biological samples.

13.2.3 Potential risks and minimization of risks

13.2.3.1 Benefits and risks

Participants will be able to obtain better immune protection against SARS-COV-2 due to a booster dose of the inactivated COVID-19 vaccine or the completion of heterologous prime-boost immunization of inactivated COVID-19 vaccine and the recombinant adenovirus vector COVID-19 vaccine. Mass vaccinations of both Inactivated SARS-CoV-2 vaccine (Vero cells) and aerosolized adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) have demonstrated good safety, so an additional dose of vaccine is not expected to result in a significant increase in safety risk. Participants enrolled in this clinical trial will not have to pay for the research vaccine. For participants in this study, they can get reasonable transportation expenses, lost income, blood collection compensation and nutrition expenses.

Vaccination may cause some AR. Common ARs include fever, tenderness at the injection site, redness and swelling. Symptoms such as dizziness, fever, muscle pain, nausea, dry mouth and fatigue may occur after inhalation. These adverse reactions are generally mild, do not require special treatment, and can be relieved or disappear by themselves. If necessary, contact the doctor for symptomatic treatment in time. AR usually resolve within 3-5 days. Immediate severe allergic reactions after vaccination are very rare, but may be life-threatening. Therefore, during the observation period of the participants in the clinic after vaccination, there will be assigned medical staff to observe and evaluate their health status. If an allergic reaction occurs, symptomatic treatment will be given as soon as possible.

In addition, ecchymosis and mild pain may occur at the site of blood samples collection. Although fainting during blood collection, and infection at the site of blood collection are very rare, it can occur.

13.2.3.2 Vaccination

Qualified inoculation consumables will be purchased, and aseptic inoculation will be performed in strict accordance with standard method to avoid AEs caused by improper inoculation or inoculation error.

If a participant experiences a grade 3 or above AR during the safety observation period, or experiences a SAE related or possibly related to the candidate vaccine, he/she should be able to receive timely medical treatment, and if necessary, the “green channel for medical treatment” should be immediately initiated for emergency treatment.

13.2.3.3 Blood specimen collection

After qualification review by the PI, the experienced nursing staff will be employed to collect venous blood samples after training as per the specified procedures to minimize pains or risks (including pain and less chance of venipuncture site infection) from which the participants suffered.

14. Access and publication of data

After the completion of this clinical trial, if the results of the trial need to be made

public and/or published, the positive results will be made public and/or published together with the negative results.