Randomized, Double-blind, Placebo-controlled Clinical Trial of Phase I/II, to Evaluate Safety and Immunogenicity of Inactivated SARS-CoV-2 Vaccine (Vero cell), in Healthy Adults Aged 18-59Years

Sponsor: Sinovac Life Sciences Co., Ltd.

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

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Statistical Analysis Plan

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Contents

1. Abbreviations

ASaT	All Subjects as Treated
BMI	Body Mass Index
DMC	Data Monitoring Committee
FAS	Full Analysis Set
GMT	Geometric Mean Titer
GMI	Geometric Mean Increase
HIV	Human Immunodeficiency Virus
ITT	Intent to Treat
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
PPS	Per Protocol Set
PT	Preferred Term
SAE	Serious Adverse event
SD	Standard Deviation
SOC	System Organ Class
SS	Safety Set
TEAE	Treatment Emergent Adverse Event

2. Purpose

To evaluate the safety and immunogenicity of inactivated SARS-CoV-2 Vaccine (Vero cell) in adults.

2.1 Clinical Trial Phase I

To evaluate the safety, tolerance, and preliminary immunogenicity of different dosage vaccine administered at different immunization schedules in adults.

2.2 Clinical Trial Phase Ⅱ

To evaluate the safety and preliminary immunogenicity of different dosage vaccine administered at different immunization schedules in adults to determine the appropriate dosage and immunization schedule for further clinical evaluation.

3. Design

3.1 Overall Design

Single centered, randomized, double-blinded, and placebo-controlled clinical trial design is adopted.

A total of 144 healthy adults aged 18~59 years old are selected as subjects in phase Ⅰ. After informed consent, subjects who pass the physical examination, meet the inclusion criteria and did not meet the exclusion criteria will be enrolled into the study. The subjects for the emergency immunization schedule (day 0, 14) and routine immunization schedule (day 0, 28) respectively, with 72 subjects for each immunization schedule. Simultaneously, subjects are phased enrolled, with 36 at medium dosage stage which will run-in first, following by 36 at high dosage stage. The subjects enrolled in each dosage stage will be randomly assigned in a 2:1 ratio to receive vaccine or placebo. The high dosage stage vaccination will start only with the condition that safety observation 0~7 days after the first dose of the medium dosage stage vaccination is finished, and the good safety profiles are confirmed by the DMC, according to the occurrence of the solicited and unsolicited adverse events, as well as the occurrence of the abnormal results of the blood routine, blood biochemistry, and urine routine testing.

The phase II clinical trial will start only with the condition that safety observation 0~7 days after the first dose of the high dosage stage vaccination is finished, and the good safety profiles are confirmed by the DMC. A total of 600 healthy adults aged 18~59 years old are selected as subjects. After informed consent, subjects who pass the physical examination, meet the inclusion criteria and didn't meet the exclusion criteria will be enrolled into the study. Subjects will receive two doses of injection at the emergency immunization schedule of day 0,14 or routine immunization schedule of day 0,28, with 300 subjects for each immunization schedule. The subjects for each schedule will be

randomly assigned in a 2:2:1 ratio to receive the medium dosage, high dosage vaccine, or placebo.

Safety Observation: The immediate reactions occur within 30 minutes after each dose of vaccination will be observed on site. The local and systemic solicited adverse events (AEs) occur within 0~7 days after each dose vaccination, as well as the unsolicited AEs from the beginning of the vaccination to 28 days after the whole schedule vaccination will be collected. Additionally, the SAEs from the beginning of the vaccination until 6 months after the whole schedule vaccination will be collected. Besides, venous blood and urine sample will be collected from all subjects in phase Ⅰ at different time points before and after vaccination for the blood routine, blood chemistry, urine routine testing, and the testing of serum inflammatory factor and antinuclear antibody, to evaluate the safety; venous blood will be collected from all subjects in phase Ⅱ at different time points before and after vaccination for the antinuclear antibody. In the safety observation, blood and urine collection at each time point are shown in Table 1.

Table 1 Blood and urine collection at each time point in safety observation

Phase and immunization schedule	Sample type	Test contents	Sampling time
Phase Ⅰ - emergency immunization schedule	Blood	Blood Routine, Blood Chemistry	Before the first dose of inoculation (D-7～D0), 3 days after the first dose of inoculation (D3), before the second dose inoculation (D14), 3 days after the second dose of inoculation (D17)
		Inflammatory Factor	Before the first dose of inoculation (D0), 7 days after the first dose of inoculation (D7), before the second dose inoculation (D14), 7 days after the second dose of inoculation (D21)
		Antinuclear Antibody	Before the first dose of inoculation (D0), 7 days after the first dose of inoculation (D7), 14 days after the first dose of inoculation (D14), 21 days after the first dose of inoculation (D21), 28 days after the first dose of inoculation (D28), 42 days after the first dose of inoculation (D42), 6 months after the whole schedule vaccination (D194)
	Urine	Urine Routine	Before the first dose of inoculation (D-7～D0), 3 days after the first dose of inoculation (D3), before the second dose inoculation (D14), 3 days after the second dose of inoculation (D17)
Phase Ⅰ - routine immunization schedule	Blood	Blood Routine, Blood Chemistry	Before the first dose of inoculation (D-7～D0), 3 days after the first dose of inoculation (D3), before the second dose inoculation (D28), 3 days after the second dose of inoculation (D31)
		Inflammatory Factor	Before the first dose of inoculation (D0), 7 days after the first dose of inoculation (D7), before the second dose inoculation (D28), 7 days after the second dose of inoculation (D35)
		Antinuclear Antibody	Before the first dose of inoculation (D0), 28 days after the first dose of inoculation (D28), 35 days after the first dose of inoculation (D35), 42 days after the first dose of inoculation (D42), 56 days after the first dose of inoculation (D56), 6 months after the whole schedule vaccination (D208)
	Urine	Urine Routine	Before the first dose of inoculation (D-7～D0), 3 days after the first dose of inoculation (D3), before the second dose inoculation (D28), 3 days after the second dose of inoculation (D31)
Phase Ⅱ - emergency immunization schedule	Blood	Antinuclear Antibody	Before the first dose of inoculation (D0), 28 days after the first dose of inoculation (D28), 42 days after the first dose of inoculation (D42), 6 months after the whole schedule vaccination (D194)
Phase Ⅱ - routine immunization schedule	Blood	Antinuclear Antibody	Before the first dose of inoculation (D0), 28 days after the whole schedule vaccination (D56), 6 months after the whole schedule vaccination (D208)

Immunogenicity observation: blood samples were collected from all subjects at different time points before and after immunization for the detection of serum neutralizing antibodies. In phase I, IgG, IgM antibodies, and specific T cell response assay (IFN-γ detection using Elispot method) were also required. In the immunogenicity observation, the blood collection at each time point is shown in Table 2.

Table2 Blood collection at different time points in immunogenicity observation

Phase and immunization schedule	Test contents	Sampling time
Phase Ⅰ - emergency immunization schedule	Neutralizing antibody, IgG, IgM	Before the first dose of inoculation (D0), 7 days after the first dose of inoculation (D7), 14 days after the first dose of inoculation (D14), 21 days after the first dose of inoculation (D21), 28 days after the first dose of inoculation (D28), 42 days after the first dose of inoculation (D42), 6 months after the whole schedule vaccination (D194)
	T cell response	Before the first dose of inoculation (D0), 14 days after the first dose of inoculation (D14), 14 days after the whole schedule vaccination (D28)
Phase Ⅰ - routine immunization schedule	Neutralizing antibody, IgG, IgM	Before the first dose of inoculation (D0), 28 days after the first dose of inoculation (D28), 35 days after the first dose of inoculation (D35), 42 days after the first dose of inoculation (D42), 56 days after the first dose of inoculation (D56), 6 months after the whole schedule vaccination (D208)
	T cell response	Before the first dose of inoculation (D0), 28 days after the first dose of inoculation (D28), 42 days after the first dose of inoculation (D42)
Phase Ⅱ - emergency immunization schedule	Neutralizing antibody	Before the first dose of inoculation (D0), 14 days after the whole schedule vaccination (D28), 42 days after the first dose of inoculation (D42), 6 months after the whole schedule vaccination (D194)
Phase Ⅱ - routine immunization schedule	Neutralizing antibody	Before the first dose of inoculation (D0), 28 days after the whole schedule vaccination (D56), 6 months after the whole schedule vaccination (D208)

This study was carried out by the Jiangsu Provincial Center for Disease Control and Prevention (Public Health Research Institute of Jiangsu Province).

3.2 Randomization and Double Blinding

3.2.1 Randomization

In phase I and phase II clinical trial, the blinding code of emergency and routine immunization schedule should be generated separately by the randomization statistician by the method of block randomization using SAS software (version 9.4). The blinding code refers to the list of the correspondence between the random number and the trial products (i.e., vaccine or placebo), which is prepared in duplicate and should be sealed after the completion of the blind coding. The original copy should be kept by the investigator for unblinding of the trial, and the duplicate copy should be kept by the sponsor. In the phase I clinical trial, the vaccine (or placebo) numbers for the emergency immunization schedule and that for the routine immunization schedule are A001-A072 and B001-B072 respectively. In the phase II clinical trial, the vaccine (or placebo) number numbers for the emergency immunization schedule and that for the routine immunization schedule are C001-C300 and D001 and D300.

The blinding code of the backup vaccine (or placebo) is also generated by the randomization statistician using SAS software (version 9.4). In the phase I clinical trial, the backup vaccine (or placebo) is prepared in a 1:1:1 ratio of medium dosage, high dosage vaccine, and placebo, and the backup vaccine (or placebo) numbers are X01-X036. In phase II clinical trial, the backup vaccine (or placebo) is prepared in a 2:2:1 ratio of medium dosage, high dosage vaccine, and placebo. In case of the circumstances such as color change and damage of the trial products, the inoculation personnel should report to the person in charge of the site and principle investigator, the initiation schedule of the backup vaccine should be started up, a backup vaccine (or placebo) number should be obtained through the online backup vaccine acquisition system, and the corresponding backup vaccine should be used instead of the problem vaccine.

3.2.2 Double Blinding

In this study, a double-blind design is adopted, in which the randomization statistician and other personnel who do not participate in the trial will engaged in vaccine (or placebo) blinding, i.e. pasting the printed number label to the specified location of the vaccine (or placebo), according to the generated blinding code. The whole process of vaccine (or placebo) blinding will be supervised by the randomization statistician. The blinding code should be sealed after the completion of the blind coding. The whole process of blinding must be recorded in writing. Personnel who conduct blinding are forbidden to participate in other relevant work of this clinical trial, and should not disclose the blinding code to any person participating in this clinical trial.

3.3 Sample Size

Clinical trial phase I: According to the requirements of the *Good Clinical Practice* and *Provisions for Drug Registration,* phase I clinical trial is a small size study aims to safety evaluation. The total sample size of phase I are 144 subjects, with a total of 72 subjects for each immunization schedule, and 48 subjects receive medium or high dosage investigational vaccine for each immunization schedule. The sample size meets the requirements of the phase I clinical trial.

Clinical trial phase II: According to the requirements of the *Good Clinical Practice* and *Provisions for Drug Registration,* the phase II clinical trial mainly evaluates the immunogenicity and safety of different dosage vaccine in the targeted subjects and the sample size is more than 300. The total number of subjects in phase II is 600, with that of subjects receive medium, high dosage vaccine and placebo os 240, 240, and 120 respectively. Thus, the subjects in the trial group are 480. The sample size meets the requirement of phase II clinical trial.

4. Clinical Trial Endpoint

4.1 Endpoint of Phase I

4.1.1 Immunogenicity Endpoint

The immunogenicity endpoints were defined on the 7th, 14th, 21st, 28th, 42nd days after the first dose of the emergency immunization schedule and the 28th, 35th, 42nd, 56th day after the first dose of the routine immunization schedule:

(1) Post-immunization neutralization antibody ≥ 4 rate: post-immunization neutralization antibody titer ≥ 1:4

(2) Post-immunization neutralizing antibody titer ⩾ 4 in the subjects with pre-immunization < 4: those whose neutralizing antibody titer was less than 1:4 before immunization, and the neutralizing antibody titer ⩾ 1:4 after immunization

(3) The 4-time increase rate of neutralizing antibody after immunization in the subjects ⩾ 4 before immunization: the subjects with ⩾ 4 before immunization was defined as those with neutralizing antibody titer ⩾ 1:4 before immunization, and those with neutralizing antibody titer increased by 4 times or more after immunization

(4) Post-immunization seroconversion rate-1: pre-immunization neutralizing antibody titer < 1:4 and post-immunization neutralizing antibody titer ⩾ 1:4; or pre-immunization neutralizing antibody titer ⩾ 1:4, post-immunization neutralizing antibody titer increased by 4 times or more than that before immunization

(5) Post-immunization neutralization antibody ≥ 8 rate: post-immunization neutralization antibody titer ≥ 1:8

(6) Post-immunization neutralizing antibody titer ⩾ 8 in the subjects with pre-immunization < 8: those whose neutralizing antibody titer was less than 1:8 before immunization, and the neutralizing antibody titer ⩾ 1:8 after immunization

(7) The 4-time increase rate of neutralizing antibody after immunization in the subjects ⩾ 8 before immunization: the subjects with ⩾ 8 before immunization was defined as those with neutralizing antibody titer ⩾ 1:8 before immunization, and those with neutralizing antibody titer increased by 4 times or more after immunization

(8) Post-immunization seroconversion rate-2: pre-immunization neutralizing antibody titer < 1:8 and post-immunization neutralizing antibody titer ≥ 1:8; or pre-immunization neutralizing antibody titer ≥ 1:8, post-immunization neutralizing antibody titer increased by 4 times or more than that before immunization

(9) Post-immunization neutralizing antibody GMT and GMI

(10) Post-immunization neutralizing antibody GMT and GMI in the subjects with pre-immunization < 4

(11) Post-immunization neutralizing antibody GMT and GMI in the subjects with post-immunization ⩾ 4

(12) Post-immunization neutralizing antibody GMT and GMI in the subjects with pre-immunization < 8

(13) Post-immunization neutralizing antibody GMT and GMI in the subjects with post-immunization ⩾ 8

(14) Post-immunization positive rate of antibody IgG: the S/CO value of antibody IgG after immunization ⩾1

(15) Post-immunization positive rate of antibody IgM: the S/CO value of antibody IgM after immunization⩾ 1

4.1.2 Exploratory Endpoint

The following exploratory endpoints were defined for emergency and routine immunization schedules, respectively:

(1) Neutralization antibody ⩾ 4 rate at 6 months after the whole schedule vaccination

(2) Neutralization antibody ⩾ 8 rate at 6 months after the whole schedule vaccination

(3) GMT of neutralizing antibody at 6 months after the whole schedule vaccination

(4) The positive rate of T cell response assay (IFN-γ detection) at 14 days after the first dose of inoculation (emergency immunization schedule) or 28 days after the first dose of inoculation (routine immunization schedule)

(5) The positive rate of T cell response assay (IFN-γ detection) at 14 days after the whole schedule vaccination

IFN-γ positive was defined as the average number of negative control < 6, the average number of peptide stimulation wells - negative control > 6; or when the average number of negative control was ⩾ 6, the average number of peptide stimulation wells / negative control points was ⩾ 2.

4.1.3 Safety Endpoint

Both emergency and routine immunization schedules included the following safety endpoints:

(1) Adverse events includes:

- Solicited adverse events: the systemic adverse events and local adverse events actively collected during the solicitation period (within 0-7 days after each dose of vaccination). Systemic adverse events include fever (axillary), acute allergic reaction, skin and mucosa abnormality, diarrhea, anorexia, vomiting, nausea, muscle pain, headache, cough, fatigue; local adverse events includes pain, induration, swelling, redness, rash, and pruritus.
- Unsolicited adverse events: Including other adverse events in the solicitation period and all adverse events in the unsolicitation period.

(2) Serious adverse event (SAE):

(3) Laboratory testing, includes:

- Blood routine: Leukocyte count, hemoglobin, platelet count;
- Blood biochemical: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), creatinine (CR), creatine phosphokinase (CPK);
- Urine routine: Urine protein (pro), urine sugar, urine red blood cell.

(4) Inflammatory factor: IL-6, IL-2, and TNF-α in serum.

(5) Antinuclear antibody: The positive rates at 7, 14, 21, 28, 42 days after the first dose of vaccination (emergency immunization schedule) and 28, 35, 42, 56 days after the first dose of vaccination (routine immunization schedule).

4.2 Endpoint of Phase II

4.2.1 Immunogenicity Endpoint

4.2.1.1 Primary Endpoint

The main immunogenicity endpoints of emergency immunization schedule and routine immunization schedule were defined as follows:

- Emergency immunization schedule: the seroconversion rate-1 at 14 days after the whole schedule vaccination;
- Emergency immunization schedule: the seroconversion rate-2 at 14 days after the whole schedule vaccination;
- Routine immunization schedule: the seroconversion rate-1 at 28 days after the whole schedule vaccination;
- Routine immunization schedule: the seroconversion rate-2 at 28 days after the whole schedule vaccination.

4.2.1.2 Secondary Endpoint

The emergency immunization schedule at 14 days after the whole schedule vaccination and the routine immunization schedule at 28 days after the whole schedule vaccination are defined as the following secondary immunogenicity endpoint:

(1) Post-immunization neutralization antibody ≥ 4 rate;

(2) Post-immunization neutralizing antibody titer ⩾ 4 in the subjects with pre-immunization < 4;

(3) The 4-time increase rate of neutralizing antibody after immunization in the subjects ⩾ 4 before immunization;

(4) Post-immunization neutralization antibody ≥ 8 rate;

(5) Post-immunization neutralizing antibody titer ⩾ 8 in the subjects with pre-immunization < 8;

(6) The 4-time increase rate of neutralizing antibody after immunization in the subjects ⩾ 8 before immunization;

(7) Post-immunization neutralizing antibody GMT and GMI;

(8) Post-immunization neutralizing antibody GMT and GMI in the subjects with pre-immunization < 4;

(9) Post-immunization neutralizing antibody GMT and GMI in the subjects with post-immunization ⩾ 4;

(10) Post-immunization neutralizing antibody GMT and GMI in the subjects with pre-immunization < 8;

(11) Post-immunization neutralizing antibody GMT and GMI in the subjects with post-immunization ⩾ 8;

The emergency immunization schedule at 28 days after the whole schedule vaccination is defined as the following secondary immunogenicity endpoint:

(12) The neutralization antibody at 28 days after the whole schedule vaccination ≥ 4 rate;

(13) The neutralizing antibody at 28 days after the whole schedule vaccination ⩾ 4 rate in the subjects with pre-immunization < 4;

(14) The 4-time increase rate of neutralizing antibody at 28 days after the whole schedule vaccination in the subjects ⩾ 4 before immunization;

(15) The seroconversion rate-1 at 28 days after the whole schedule vaccination

(16) The neutralization antibody ≥ 8 rate at 28 days after the whole schedule vaccination;

(17) The neutralizing antibody at 28 days after the whole schedule vaccination ⩾ 8 in the subjects with pre-immunization < 8;

(18) The 4-time increase rate of neutralizing antibody at 28 days after the whole schedule vaccination in the subjects ⩾ 8 before immunization;

(19) The seroconversion rate-2 at 28 days after the whole schedule vaccination

(20) The neutralizing antibody GMT and GMI at 28 days after the whole schedule vaccination;

(21) The neutralizing antibody GMT and GMI at 28 days after the whole schedule vaccination in the subjects with pre-immunization < 4;

(22) The neutralizing antibody GMT and GMI at 28 days after the whole schedule vaccination in the subjects with

post-immunization ⩾ 4;

(23) The neutralizing antibody GMT and GMI at 28 days after the whole schedule vaccination in the subjects with pre-immunization < 8;

(24) The neutralizing antibody GMT and GMI at 28 days after the whole schedule vaccination in the subjects with post-immunization ⩾ 8;

4.2.2 Exploratory Endpoint

The exploratory endpoints of emergency immunization schedule and routine immunization schedule were defined as follows:

(1) Neutralization antibody ⩾ 4 rate at 6 months after the whole schedule vaccination

(2) Neutralization antibody ⩾ 8 rate at 6 months after the whole schedule vaccination

(3) GMT of neutralizing antibody at 6 months after the whole schedule vaccination

4.2.3 Safety Endpoint

The safety endpoints of emergency immunization schedule and routine immunization schedule were as follows:

(1) Adverse events includes:

- Solicited adverse events: the systemic adverse events and local adverse events actively collected during the solicitation period (within 0-7 days after each dose of vaccination). Systemic adverse events include fever (axillary), acute allergic reaction, skin and mucosa abnormality, diarrhea, anorexia, vomiting, nausea, muscle pain, headache, cough, fatigue; local adverse events includes pain, induration, swelling, redness, rash, and pruritus.
- Unsolicited adverse events: Including other adverse events in the solicitation period and all adverse events in the unsolicitation period.

(2) Serious adverse event (SAE);

(3) Antinuclear antibody: The positive rates at 28, 42 days after the first dose of vaccination (emergency immunization schedule), and 56 days after the first dose of vaccination (routine immunization schedule). Positive was defined as antinuclear antibody concentration ⩾ 10U/ml.

5. Analysis Set

Full Analysis Set (FAS): A subjects defined according to the principle of intent analysis (ITT), including all the subjects who have been randomized, have received at least one dose of vaccination, have finished blood collection before/after vaccination for at least one time with the corresponding antibody assay results provided. The subjects who are vaccinated with the wrong vaccine will be kept in the originally assigned group according to the ITT principle, for the immunogenicity evaluation.

Per-Protocol Set (PPS): A subset of FAS, including all the subjects who meet the inclusion criteria and don't meet the exclusion criteria, have been randomized, have finished the full-course vaccination within the protocol required time window, and have finished the blood collection before/after vaccination with the corresponding antibody assay results provided. Subjects who meet the following conditions are not allowed to enter PPS:

(1) Those who violate the test scheme;

(2) Those who are vaccinated with the wrong vaccine;

(3) Use of protocol prohibited vaccines or drugs:

- Other investigational or unlicensed products (drugs or vaccines)
- long-term use (lasting more than 14 days) of immunosuppressive or other immunomodulatory drugs (inhaled or topical steroids are allowed)

- Immunoglobulin and/or blood preparations

(4) Newly diagnosed autoimmune diseases, including human immunodeficiency virus (HIV) infection;

(5) Other situations affecting the evaluation of vaccine immunogenicity.

Immune Persistence Set (IPS): All subjects who had completed the whole schedule vaccination and had effective antibody titer value at 6 months after the whole schedule vaccination were included.

The above Full Analysis Set, Per-Protocol Set, and Immune Persistence Set are defined for emergency immunization schedule and routine immunization schedule in phases I and II.

Safety Analysis Set (Safety Set, SS): All randomized subjects with at least one dose of the study vaccine were included. Among them, according to ASaT (All Subjects as Treated) principle, the safety evaluation was carried out according to the actual vaccination group.

- The safety set is mainly used for the safety evaluation of the vaccine, and the safety set of each dose is used for the safety evaluation of each dose.
- The safety set is divided into total safety set, first dose safety set, and second dose safety set. The safety analysis of each dose was based on the actual number of vaccinated people. The first dose of safety set includes all subjects who have completed the first dose of vaccination, which are recorded as SS1; the second dose of safety set includes all subjects who have completed the second dose of vaccination and are recorded as SS2.

The above analysis sets will be discussed and decided by the main researcher, the sponsor, the statistician, and the data manager before the database is locked and in the data blind audit process.

6. Statistical Analysis Method

6.1 General Principles

Quantitative data will be summarised descriptively, including mean, median, standard deviation, minimum, and maximum. The categorical data or ordinal data will be summarised by count and proportion.

All statistical analysis will conduct with SAS 9.4.

6.2 Enrollment and completion

The number of subjects screened, randomized, complete, and enter the analysis subjects will be provided by group per phase and immunization schedule. The reasons to discontinue from the study will be summarised. The subjects, who screen failure, discontinue from the study and excluded from the analysis subjects, will be listed respectively.

6.3 Demographics and the Baseline Characteristics

Summaries of the following demographics and other baseline characteristics will be presented by group:

- Age, gender and race
- Physical examination, including temperature, height, weight and BMI,
- IgG and IgM at baseline,
- Baseline nucleic acid testing result,
- Whether it has passed the laboratory screening before enrollment

ANOVA will be used to detect the difference for age, temperature, height, weight, and BMI among groups, and group t-test will be used for those between immunization schedules. Chi-square test or Fisher exact test will be used to detect the difference for gender, race, antibody screening results, nucleic acid testing result, and whether it has passed the laboratory screening before enrollment among groups and between immunization schedules.

Descriptive statistics for the compliance will be provided by group, including whether complete all visits, whether complete all vaccinations, whether complete all safety observations, and whether complete the immunogenicity blood collections. Chi-square test or Fisher exact test will be used to detect the difference among groups and between

immunization schedules.

The counts and proportions of concomitant medication and concomitant vaccine will be summarised by group per phase and immunization schedule. Fisher exact test will be used to detect the difference among groups and between immunization schedules. Both concomitant medication and concomitant vaccines will be listed.

6.4 Immunogenicity Analysis

(1) The statistical method for immunogenicity evaluation for emergency immunization schedule and routine immunization schedule in Phase I will be carried out as follows.

Taking emergency immunization schedule in Phase I as an example, the following rates will be calculated by the group and their confidence intervals will be estimated by Clopper-Pearson method:

- Rate of serum neutralizing antibody titer no less than 4 at 7 days after the first dose,
- Rate of serum neutralizing antibody titer no less than 4 at 7 days after the first dose in the subjects with antibody titer before vaccination less than 4,
- Quadruple increase rate of serum neutralizing antibody titer at 7 days after the first dose in the subjects with antibody titer before vaccination no less than 4,
- Serum neutralizing seroconversion rate-1 (bounded by 4) at 7 days after the first dose,
- Rate of serum neutralizing antibody titer no less than 8 at 7 days after the first dose in the subjects with antibody titer before vaccination less than 8,
- Quadruple increase rate of serum neutralizing antibody titer at 7 days after the first dose in the subjects with antibody titer before vaccination no less than 8,
- Serum neutralizing seroconversion rate-2 (bounded by 8) at 7 days after the first dose,
- IgG antibody positive rate at 7 days after the first dose,
- IgM antibody positive rate at 7 days after the first dose.

Chi-square test or Fisher exact test will be used to detect differences among groups per rate as above. Furthermore, if statistically significance exists, the pairwise comparison will be conducted between groups by Chi-square test or Fisher exact test.

Descriptive statistics for the following serum neutralizing antibody geometric mean titers (GMTs) and geometric mean increasements (GMIs), including geometric mean and 95% confidence interval (95% CI), will be provided by group:

- GMT and GMI at 7 days after the first dose,
- GMT and GMI at 7 days after the first dose in the subjects with antibody titer before vaccination less than 4,
- GMT and GMI at 7 days after the first dose in the subjects with antibody titer before vaccination no less than 4,
- GMT and GMI at 7 days after the first dose in the subjects with antibody titer before vaccination less than 8,
- GMT and GMI at 7 days after the first dose in the subjects with antibody titer before vaccination no less than 8.

ANOVA model with log-transformation (per GMT and GMI as above) will be used to detect the difference among groups. Furthermore, if statistically significance exists, pairwise comparison will be conducted between groups by group t-test with log-transformation. Inversetiter distributions of antibody before vaccination and at 7 days after the first dose will be plotted by group, respectively.

The statistical analysis method of immunogenicity evaluation on 14, 21, 28, 42 days after the first dose immunization of phase I emergency immunization schedule and 28, 35, 42, 56 days after the first dose immunization of phase I

routine immunization schedule was the same as that of 7 days after the first dose in emergency immunization schedule of phase I as above. Additionally, using the same method, immunogenicity comparison between emergency immunization schedule and routine immunization schedule in Phase I will be conducted by group (medium-dose group and high-dose group) at 14 days and 28 days after the whole schedule vaccination, respectively. 14 days after the whole schedule vaccination is 28 days after their first dose in emergency immunization schedule and 42 days after the first dose in routine immunization schedule, respectively. Meanwhile, 28 days after the whole schedule vaccination is 42 days after their first dose in emergency immunization schedule and 56 days after the first dose in routine immunization schedule, respectively.

(2) The statistical method for immunogenicity evaluation for emergency immunization schedule and routine immunization schedule in Phase II will be carried out as follows.

Taking emergency immunization schedule in Phase II as an example, the following rates will be calculated by the group and their confidence intervals will be estimated by Clopper-Pearson method:

- Rate of serum neutralizing antibody titer no less than 4 at 14 days after the whole schedule vaccination,
- Rate of serum neutralizing antibody titer no less than 4 at 14 days after the whole schedule vaccination in the subjects with antibody titer before vaccination less than 4,
- Quadruple increase rate of serum neutralizing antibody titer at 14 days after the whole schedule vaccination in the subjects with antibody titer before vaccination no less than 4,
- Serum neutralizing seroconversion rate-1 (bounded by 4) 14 days after the whole schedule vaccination,
- Rate of serum neutralizing antibody titer no less than 8 14 days after the whole schedule vaccination in the subjects with antibody titer before vaccination less than 8,
- Quadruple increase rate of serum neutralizing antibody titer14 days after the whole schedule vaccination in the subjects with antibody titer before vaccination no less than 8,
- Serum neutralizing seroconversion rate-2 (bounded by 8) 14 days after the whole schedule vaccination,
- IgG antibody positive rate 14 days after the whole schedule vaccination,
- IgM antibody positive rate 14 days after the whole schedule vaccination.

Chi-square test or Fisher exact test will be used to detect differences among groups per rate as above. Furthermore, if statistically significance exists, pairwise comparison will be conducted between groups by Chi-square test or Fisher exact test.

Descriptive statistics for the following serum neutralizing antibody geometric mean titers (GMTs) and geometric mean increasements (GMIs), including geometric mean and 95% CI, will be provided by group:

- GMT and GMI at 14 days after the whole schedule vaccination,
- GMT and GMI at 14 days after the whole schedule vaccination in the subjects with antibody titer before vaccination less than 4,
- GMT and GMI at 14 days after the whole schedule vaccination in the subjects with antibody titer before vaccination no less than 4,
- GMT and GMI at 14 days after the whole schedule vaccination in the subjects with antibody titer before vaccination less than 8,
- GMT and GMI at 7 days after the whole schedule vaccination in the subjects with antibody titer before vaccination no less than 8.

ANOVA model with log-transformation (per GMT and GMI as above) will be used to detect the difference among groups. Furthermore, if statistically significance exists, group t-test with log-transformation will be conducted between groups. Inversetiter distributions of antibody before vaccination and at 14 days after the whole schedule vaccination will be plotted by group, respectively.

The statistical analysis method of immunogenicity evaluation of phase II emergency immunization schedule and 28

days after the whole schedule vaccination in routine immunization schedule was the same as that of phase II emergency immunization schedule 14 days after the whole immunization as above. Additionally, The immunogenicity of medium-dose group and high-dose group in phase II was compared among different immunization schedules. The statistical analysis method was the same as that of the phase II emergency immunization schedule 14 days after the whole immunization.

6.5 Exploratory Analysis

(1) The statistical method for exploratory evaluation for emergency immunization schedule and routine immunization schedule in Phase I is summarised as follows.

The following rates will be calculated by the group and their confidence intervals will be estimated by the Clopper-Pearson method:

- Rate of serum neutralizing antibody titer no less than 4 at 6 months after the whole schedule vaccination,
- Rate of serum neutralizing antibody titer no less than 8 at 6 months after the whole schedule vaccination,
- Positive rate of T cell response (IFN-γ detection using Elispot) at 14/28 days after the first dose in emergency immunization schedule/ routine immunization schedule,
- Positive rate of T cell response (IFN-γ detection using Elispot) at 14 days after the whole schedule vaccination,

Chi-square test or Fisher exact test will be used to detect differences among groups per rate as above. Furthermore, if statistically significant, pairwise comparison will be conducted between groups by Chi-square test or Fisher exact test. Additionally, using the same method, their comparison between emergency immunization schedule and routine immunization schedule will be conducted by group (medium-dose group and high-dose group), respectively.

Descriptive statistics for the difference between the average number of stimulation holes for T cell response assay (IFN-γ detection using Elispot method) and that of negative control on day 14 (emergency immunization schedule) / 28 (routine immunization schedule) after the first dose of immunization will be provided by group. Rank-sum test will be used to test the difference among groups. Furthermore, if statistically significance exists, pairwise comparison will be conducted between groups by rank-sum test.

Descriptive statistics for GMT at 6 months after the whole schedule vaccination, including geometric mean and 95 confidence interval (95% CI), will be provided by the group. Furthermore, if statistically significance exists, group t-test with log-transformation will be conducted between groups. Additionally, using the same method, T cell response assay (IFN-γ detection using Elispot method) comparison between emergency immunization schedule and routine immunization schedule will be conducted by group (medium-dose group and high-dose group), respectively.

(2) The statistical method for exploratory evaluation for emergency immunization schedule and routine immunization schedule in Phase II is summarised as follows.

The following rates of antibody will be calculated by the group and their confidence intervals will be estimated by the Clopper-Pearson method:

- Rate of serum neutralizing antibody titer no less than 4 at 6 months after the whole schedule vaccination,
- Rate of serum neutralizing antibody titer no less than 8 at 6 months after the whole schedule vaccination,

Chi-square test or Fisher exact test will be used to detect differences among groups per rate as above. Furthermore, if statistically significant, pairwise comparison will be conducted between groups by Chi-square test or Fisher exact test.

GMT at 6 months after the whole schedule vaccination will be calculated by the group in terms of geometric mean and 95% CI. ANOVA model with log-transformation will be used to detect the difference among groups. Furthermore, if statistically significance exists, group t-test with log-transformation will be conducted between groups.

6.6 Safety Analysis

6.6.1 Adverse Events

All AEs and SAEs will be coded using Medical Dictionary for Regulatory Activities and will be analyzed in terms of system organ class (SOC) and preferred term (PT). Additionally, solicited AE will be analyzed in terms of systemic AE and local AE, which are predefined in the protocol. This study mainly analyzes Treatment Emergent Adverse Event (TEAE), which occurs after vaccination. The AEs occur before vaccination will only be displayed in the listing. Unless particularly stated, hereinafter all of the AEs are TEAE.

AE will be summarised per phase and immunization schedule as follows: The counts and proportion for AE, related AE, and unrelated AE will be summarised. Fisher exact test will be used to test the difference among groups. Besides, AE and related AE will be analyzed according to the severity, dose distribution, and occurrence time. Related and unrelated AE will be listed, respectively.

AE after every dose will be summarized using the Safety Set for the corresponding dose, respectively. AE occurrence will be compared between immunization schedules by group.

The counts and proportions for SAE, related SAE, and unrelated SAE will be summarised by the group. Fisher exact test will be used to test the difference among groups. SAE will be listed.

6.6.2 Laboratory Test

Laboratory parameters for emergency immunization schedule and routine immunization schedule in Phase I will be analyzed in the following way.

Laboratory parameters at each time point and change from baseline to each post-baseline time point will be summarized using descriptive summary statistics for each parameter by group, and ANOVA will be used to detect the difference among groups. Group t-test will be used to detect the difference between immunization schedules by group.

Additionally, the counts and frequency of laboratory clinically significances will be calculated for each parameter and visit by group. Fisher exact test will be used to detect the difference for each parameter and visit among groups. Shift Tables from pre-vaccination to post-vaccination will be provided for each laboratory parameter by the group.

Also, the occurrence of clinically significant abnormal laboratory results within a trial subject will be analyzed using descriptive summary statistics for each parameter and visit by group.

6.6.3 Antinuclear Antibody

(1) The statistical method for antinuclear antibody evaluation for emergency immunization schedule and routine immunization schedule in Phase I will be carried out as follows.

The positive rate of antinuclear antibody was calculated by group on the 7th day after the first dose of immunization in phase I emergency immunization schedule, and their confidence intervals will be estimated by the Clopper-Pearson method. A Chi-square test or Fisher exact test will be used to detect differences among groups. Furthermore, if statistically significance exists, the pairwise comparison will be conducted between groups by Chi-square test or Fisher exact test.

Descriptive statistics in each group for the antinuclear antibody geometric mean titers (GMTs) and geometric mean increasements (GMIs) at 7th day after the first dose of immunization in phase I emergency immunization schedule, including geometric mean and 95% confidence interval (95% CI), will be provided by group. ANOVA model with log-transformation (per GMT and GMI as above) will be used to detect the difference among groups.

The statistical analysis method of antinuclear antibody evaluation on 14, 21, 28, 42 days after the first dose immunization of phase I emergency immunization schedule and 28, 35, 42, 56 days after the first dose immunization of phase I routine immunization schedule was the same as that of 7 days after the first dose in emergency immunization schedule of phase I.

Additionally, using the same method, the positive rate of antinuclear antibody comparison between emergency immunization schedule and routine immunization schedule in Phase I will be conducted by group (medium-dose group and high-dose group) at 14 days and 28 days after the whole schedule vaccination, respectively. 14 days after the whole schedule vaccination is 28 days after their first dose in emergency immunization schedule and 42 days after the first dose in routine immunization schedule, respectively. Meanwhile, 28 days after the whole schedule vaccination is 42 days after their first dose in emergency immunization schedule and 56 days after the first dose in routine immunization schedule, respectively.

(2) The statistical method for antinuclear antibody evaluation for emergency immunization schedule and routine immunization schedule in Phase II will be carried out as follows.

The positive rate of antinuclear antibody was calculated by group on the 28th day after the first dose of immunization in phase II emergency immunization schedule, and their confidence intervals will be estimated by the Clopper-Pearson method. A Chi-square test or Fisher exact test will be used to detect differences among groups. Furthermore, if statistically significance exists, the pairwise comparison will be conducted between groups by Chi-square test or Fisher exact test.

Descriptive statistics in each group for the antinuclear antibody geometric mean titers (GMTs) and geometric mean increasements (GMIs) at 7th day after the first dose of immunization in phase II emergency immunization schedule, including geometric mean and 95% confidence interval (95% CI), will be provided by group. ANOVA model with log-transformation (per GMT and GMI as above) will be used to detect the difference among groups.

The statistical analysis method of antinuclear antibody evaluation on 42 days after the first dose immunization of phase II emergency immunization schedule and 56 days after the first dose immunization of phase II routine immunization schedule was the same as that of 28 days after the first dose in emergency immunization schedule of phase II.

Chi-square test or Fisher exact test will be used to detect differences of positive rate of antinuclear antibody between the middle dose group and the high-dose group at 28 days after the whole immunization in phase Ⅱ. Additionally, using the same method, the positive rate of antinuclear antibody comparison between emergency immunization schedule and routine immunization schedule in Phase II will be conducted by group (medium-dose group and high-dose group) at 14 days and 28 days after the whole schedule vaccination, respectively. 14 days after the whole schedule vaccination is 28 days after their first dose in emergency immunization schedule and 42 days after the first dose in routine immunization schedule, respectively.

6.6.4 Inflammatory Factors

The statistical method for inflammatory factors evaluation for emergency immunization schedule and routine immunization schedule in Phase I will be carried out as follows.

The changes of IL-6, IL-2, and TNF-α comparison between emergency immunization schedule and routine immunization schedule will be conducted by group (medium-dose group, high-dose group and placebo group) at 7 days after each dose were statistically described, and the difference among groups was analyzed by Rank-sum test.

6.7 Multiplicity problem

This study is a phase I / II exploratory study, so multiple adjustments are not considered. In the results of immunogenicity evaluation and safety evaluation, the calculated p-value is only nominal p-value, which is mainly used to describe the strength of association between evaluation endpoint and treatment group, but not as the basis for formal statistical inference.

6.8 Subgroup analysis

Subgroup analysis was not planned for this trial.

6.9 Processing of missing data

Concerning the statistical analysis of FAS, the missing data of the post-vaccination antibody test result will be filled

by the method of Last Observation Carried Forward (LOCF). The missing data of the pre-vaccination antibody result will be filled with the maximum of pre-vaccination antibody results among all the subjects. In terms of the evaluation of the immunogenicity, exploratory, and safety endpoints, missing data will not be filled.

6.10 Interim analysis

The safety interim analysis will be separately conducted after 7 days of safety observation after the first dose of vaccination of phase I and 28 days after the whole schedule vaccination of phase II.

7. Notes on the plan

This analysis plan was drafted based on the relevant description in the study protocol and defined the endpoints of immunogenicity, exploratory, and safety evaluation. According to the basic characteristics of each index in the protocol, combined with the specific requirements of this study, the specific statistical analysis method of the relevant evaluation endpoint is proposed. Considering that there may be some unexpected changes in the final data distribution form of clinical trials, the statistical analysis method may be slightly adjusted, and the presentation of the corresponding statistical analysis results may also change to a certain extent.

The table of statistical analysis of this analysis plan will be provided separately in the form of an appendix.

Version history

Version	Date	Writer	Update
V1.0	2020-06-05	Qun Shu, Guoqing Zhao	Initial version