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Supplementary appendix

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Supplement to: Chen G-L, Li X-F, Dai X-H, et al. Safety and immunogenicity of the SARS-CoV-2 ARCoV mRNA vaccine in Chinese adults: a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet Microbe* 2022; published online Jan 24. https://doi.org/10.1016/S2666-5247(21)00280-9.

Supplementary appendix 1

Supplement to: Chen G, Li X, Dai X, et al. Safety and immunogenicity of the SARS-CoV-2 ARCoV mRNA vaccine in Chinese adults: a randomized, double-blind, placebo-controlled, phase 1 trial.

此简体中文译文由作者提交，我方按照提供的版本刊登。此译文并未经过同行审阅。医学期刊《柳叶刀》的编辑流程仅适用于英文原稿，英文原稿应作为此手稿的参考。

Appendix 1

摘要

背景： 由新冠病毒 SARS-CoV-2 感染导致的 COVID-19 大流行仍在席卷全球，迫切需要安全有效的疫苗结束这场疫情。ARCoV 是一种编码 SARS-CoV-2 刺突蛋白受体结合域（RBD）的 mRNA 疫苗。本研究旨在初步评估 ARCoV 的安全性、耐受性和免疫原性。

方法： I 期临床试验采用单中心、随机、双盲、剂量递增、安慰剂对照的试验方案，在中国浙江省杭州市树兰（杭州）医院完成。I 期临床试验纳入了 120 位 18 到 59 岁健康成年参与者，无新冠感染和密接史。以剂量递增的方式（5 μg, 10 μg, 15 μg, 20 μg, 25 μg），受试者随机接受两剂经肌肉注射疫苗或安慰剂，两剂间隔 28 天。通过使用标准 ELISA 检测抗 SARS-CoV-2 RBD IgG 抗体，以及基于假病毒和活病毒中和试验的中和抗体，全面评估受试者在疫苗接种后的体液免疫反应。采用酶联免疫斑点法评估 SARS-CoV-2 RBD 特异的 T 细胞反应，包括 IFN-γ 和 IL-2 细胞因子的产生。I 期临床试验的主要安全性结果是疫苗接种后 56 天内发生的不良事件。次要安全性结果为实验室检测指标的异常，次要免疫原性结果为体液免疫反应，探索性免疫原性结果为病毒特异性的 T 细胞反应。I 期临床试验在 www.chictr.org.cn 网站的注册号是：ChiCTR2000039212.

结果： 在 I 期临床试验中，120 位合格参与者(男性 62.5%，女性 37.5%；平均年龄 27.0 岁)被随机分配接受 5 个剂量的疫苗或安慰剂（每组 20 人）。120 位参与者均接种了首剂疫苗，其中 117 人接受了第二剂加强免疫。在免疫后 56 天内，所有受试者均未发生严重不良事件，大部分为轻度或中度不良事件。发热是最常见的全身不良反应。疫苗组三级主要不良事件的例数分别是 5 μg 组：0 (0%)，10 μg 组：3 (15%)，15 μg 组：6 (30%)，20 μg 组：7 (35%)，25 μg 组：5 (31%)及安慰剂组：0 (0%)。95%以上的发热在接种疫苗后的 2 天内消退。值得注意的是，在本研究中，ARCoV 在首剂和加强免疫后的全身不良事件发生率相似。抗 RBD IgG 和中和抗体等体液免疫反应在增强免疫后 7 天显著增加，并在增强免疫后 14~28 天达到高峰。 特异性 T 细胞反应在完全接种后 7~14 天达到高峰。 其中，15 μg 的中和抗体水平最高，约为新冠肺炎恢复期患者的 2 倍。

解释： mRNA 疫苗 ARCoV 在所有五个剂量水平上都具有良好的耐受性和安全性。ARCoV 可接受的安全性，以及诱导产生的强烈的体液和细胞免疫应答，支

持进一步大规模的 ARCoV 临床试验。

资助： 国家重点研发项目、军事科学院特别资助、国家杰出青年基金、国家自然科学基金创新团队、中国医学科学院医学创新基金。

Supplementary appendix 2

Supplement to: Chen G, Li X, Dai X, et al. Safety and immunogenicity of the SARS-CoV-2 ARCoV mRNA vaccine in Chinese adults: a randomized, double-blind, placebo-controlled, phase 1 trial.

Appendix 2

Content

Inclusion and exclusion criteria

Table 1: Inclusion and exclusion criteria for participants screening

Item	Criteria
Inclusion criteria	Healthy adults aged 18-59 years; informed consent; good compliance; axillary temperature <37.3 ℃
Exclusion criteria	Pulse <50 or >100 beats/min; SBP≥140 mmHg or DBP≥90 mmHg; BMI <18 or >30 kg/m^2; clinical screening laboratory evaluations are 1.2 times the normal reference range
	Pregnancy or lactation or be menstruating or egg or sperm donation
	A history of COVID vaccination
	A history of infection with SARS-CoV-2, SARS-CoV or MERS-CoV or suspected cases; a history of travelling to high outbreak areas or regions outside of China; any history of serious adverse reactions to vaccines or drugs
	Positive for SARS-CoV-2 nucleic acid test and serum-specific IgM/IgG antibodies against SARS-CoV-2
	Positive for hepatitis B surface antigen, hepatitis C virus core antigen, hepatitis C virus antibody, Treponema pallidum specific antibody or HIV types 1 or 2 antibodies
	In acute diseases or in the acute onset of chronic diseases, or axillary temperature ≥37.3℃ or symptoms of upper respiratory tract infection within 7 days before the first dose of vaccine
	A history of vaccination 1 month before the first dose
	A history of needle and blood sickness or inability to tolerate venipuncture
	A hereditary bleeding tendency or coagulation dysfunction; a history of thrombosis or bleeding; abnormal results of coagulation function related indicators
	Congenital or acquired immunodeficiency
	No spleen or functional no spleen or surgical removal of other vital organs
	Serious diseases with previous clinical manifestations (exclude chronic history with stable control, such as diabetes, hypertension, etc)
	A history of surgery within 3 months prior to signing the informed consent, or planned to have surgery during or within 3 months after the end of the study
	A history of donation or blood loss (≥450 mL) within 3 months prior to signing the informed consent; received blood or blood product; blood donation planning during the trial
	A history of any investigational or unregistered product (drug, vaccine, biological product or device) other than the study vaccine, or planned to be used during the study period, within 3 months prior to signing the informed consent
	A history of immunosuppressive therapy within 6 months prior to signing the informed consent
	Other circumstances not suitable for participating in the trial

Table 1-2 Clinical characteristics of the convalescent COVID-19 patients.

Patient #	Sex	Age(years)	Diagnosis	Sampling time	CCID50
1	Female	37	mild	6	45
2	Female	29	mild	6	91
3	male	32	mild	4	16
4	male	35	mild	6	91
5	male	68	mild	6	91
6	male	32	mild	4	45
7	male	32	mild	4	128
8	male	64	mild	16	45
9	male	37	mild	11	91
10	male	48	severe	10	45
11	male	37	mild	12	64
12	Female	72	mild	12	64
13	male	48	severe	7	45
14	male	37	mild	8	91
15	male	37	mild	7	91
16	Female	72	mild	10	32
17	Female	53	mild	12	91
18	male	44	mild	12	91
19	male	48	severe	15	64
20	Female	53	mild	16	91
21	male	66	mild	13	91
22	Female	72	mild	6	64
23	male	32	mild	12	91
24	male	37	mild	15	32
25	male	40	severe	17	91
26	male	59	severe	12	32
27	Female	53	mild	9	91
28	male	37	mild	6	64
29	male	75	severe	16	256
30	Female	53	mild	7	91
31	male	64	mild	10	91
32	male	36	severe	7	64
33	male	36	severe	15	91
34	male	64	mild	8	181

Notes: Sampling time, weeks after symptom onset

Adverse event

Table 2-1: Toxicity grading scales for solicited systemic and local adverse events by NMPA

	Grade 1	Grade 2	Grade 3
Local Reaction			
Pain	Does not interfere or slightly interfere with activity	Interferes with activity	Prevents daily activity
Induration/swelling (diameter)	Diameter: 2.5-5 cm or area: 6.25-25 cm^2 and Does not interfere or slightly interfere with activity	Diameter: 5.1-10 cm or area: 25-100 cm^2 or interferes with activity	Diameter: ≥10 cm or area: ≥100 cm^2 or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or prevents daily activity
Erythema/redness (diameter)	Diameter: 2.5-5 cm or area: 6.25-25 cm^2 and Does not interfere or slightly interfere with activity	Diameter: 5.1-10 cm or area: 25-100 cm^2 or interferes with activity	Diameter: ≥10 cm or area: ≥100 cm^2 or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or prevents daily activity
Itch	Releases spontaneously, or <48 h after treatment	No remission within 48 h after treatment	Prevents daily activity
Systemic Reaction			
Fever (axillary, °C)	37.3-38.0	38.0-38.5	38.5-39.5
Diarrhea	Mild or transient, 3-4 times/day, abnormal fecal characteristics, or mild diarrhea lasting less than 1 week	Moderate or persistent, 5-7 times/day, abnormal fecal traits, or diarrhea with > for 1 week	>7 times/day, abnormal fecal characteristics, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, and intravenous infusion of >2L
Nausea	Transient (<24 h) or intermittent and generally normal intake of food	Persistent nausea leads to reduced food intake (24-48 h)	Persistent nausea results in almost no food intake (>48 h) or the need for intravenous rehydration
Vomiting	1-2 episodes/ 24 h, no interference with activity	3-5 episodes/ 24 h or some interference with activity	>6 episodes/ 24 h or requires IV hydration
Headache	No interference with activity and do not require treatment	Transient, slightly affecting daily activities, may require treatment or intervention	Prevents daily activity and require treatment or intervention
Muscle pain	No interference with activity	Slightly affecting daily activities	Severe and prevents daily activity
Joint pain	Mild and no interference with activity	Moderate pain; need analgesics and/or pain that interferes with function but does not interfere with daily activities	Severe pain; needs analgesics and/or interferes with daily activities
Chills	Slightly cold, teeth chattering	Moderate tremors throughout the body	Severe/persistent shaking
Fatigue/Malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity and inability to work
Acute allergic reaction	Local urticaria (blisters) and do not require treatment	Local urticaria, requiring treatment or mild angioedema, without treatment	Extensive urticaria or angioedema requires treatment or mild bronchospasm

Table 2-2: Toxicity grading scales for solicited systemic and local adverse events by FDA

	Grade 1	Grade 2	Grade 3
Local reaction			
Pain	Does not interfere with activity	Repeated use of nonnarcotic pain reliever >24 h/ interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Erythema/Redness	2.5-5 cm	5.1-10 cm	>10 cm
Induration/Swelling	2.5-5 cm and does not interfere with activity	5.1-10 cm/interferes with activity	>10 cm/prevents daily activity
Vital Signs			
Fever (oral, °C)	38.0-38.4	38.5-38.9	39.0-40
Tachycardia-beats/min	101-115	116-130	>130
Bradycardia-beats/min	50-54	45-49	<45
Hypertension SBD (mm Hg)	141-150	151-155	>155
Hypertension DBP (mm Hg)	91-95	96-100	>100
Hypotension SBD (mm Hg)	85-89	80-84	<80
Respiratory Rate-breaths/min	17-20	21-25	>25
Systemic Reaction			
Nausea/vomiting	No interference with activity / 1-2 episodes/ 24 h	Some interference with activity /> 2 episodes/ 24 h	Prevents daily activity, requires outpatient IV hydration
Diarrhea	2-3 loose stools/ <400 gms/ 24 h	4-5 stools/ 400-800 gms/ 24 h	6 or more watery stools/ >800 gms/ 24 h / requires outpatient IV hydration
Headache	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 h/ some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity
Illness or clinical adverse event	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention

Note: ER: Emergency room

Table 2-3: Unsolicited adverse events classified by organ class and investigator-assigned relationship to study vaccination by day 56

System organ class	Severity	Related to vaccination	Not related to vaccination
Infections	Grade 1	18 (15%)	2 (1.7%)
	Grade 2	2 (1.7%)	3 (2.5%)
	Grade 3	-	-
Nervous system disorders	Grade 1	-	-
	Grade 2	-	2 (1.7%)
	Grade 3	-	-
Injury, poisoning, and procedural complications	Grade 1	-	1 (0.8%)
	Grade 2	-	-
	Grade 3	-	1 (0.8%)
Respiratory, thoracic and mediastinal disorders	Grade 1	3 (2.5%)	-
	Grade 2	2 (1.7%)	1 (0.8%)
	Grade 3	-	-
Skin and subcutaneous tissue disorders	Grade 1	-	-
	Grade 2	-	1 (0.8%)
	Grade 3	-	-
General disorders and administration site conditions	Grade 1	1 (0.8%)	-
	Grade 2	2 (1.7%)	-
	Grade 3	-	-
Kidney and urinary system diseases	Grade 1	11 (9.2%)	-
	Grade 2	2 (1.7%)	-
	Grade 3	-	-
Gastrointestinal disorders	Grade 1	1 (0.8%)	2 (1.7%)
	Grade 2	-	1 (0.8%)
	Grade 3	-	-
Heart organ disease	Grade 1	1 (0.8%)	-
	Grade 2	-	-
	Grade 3	-	-

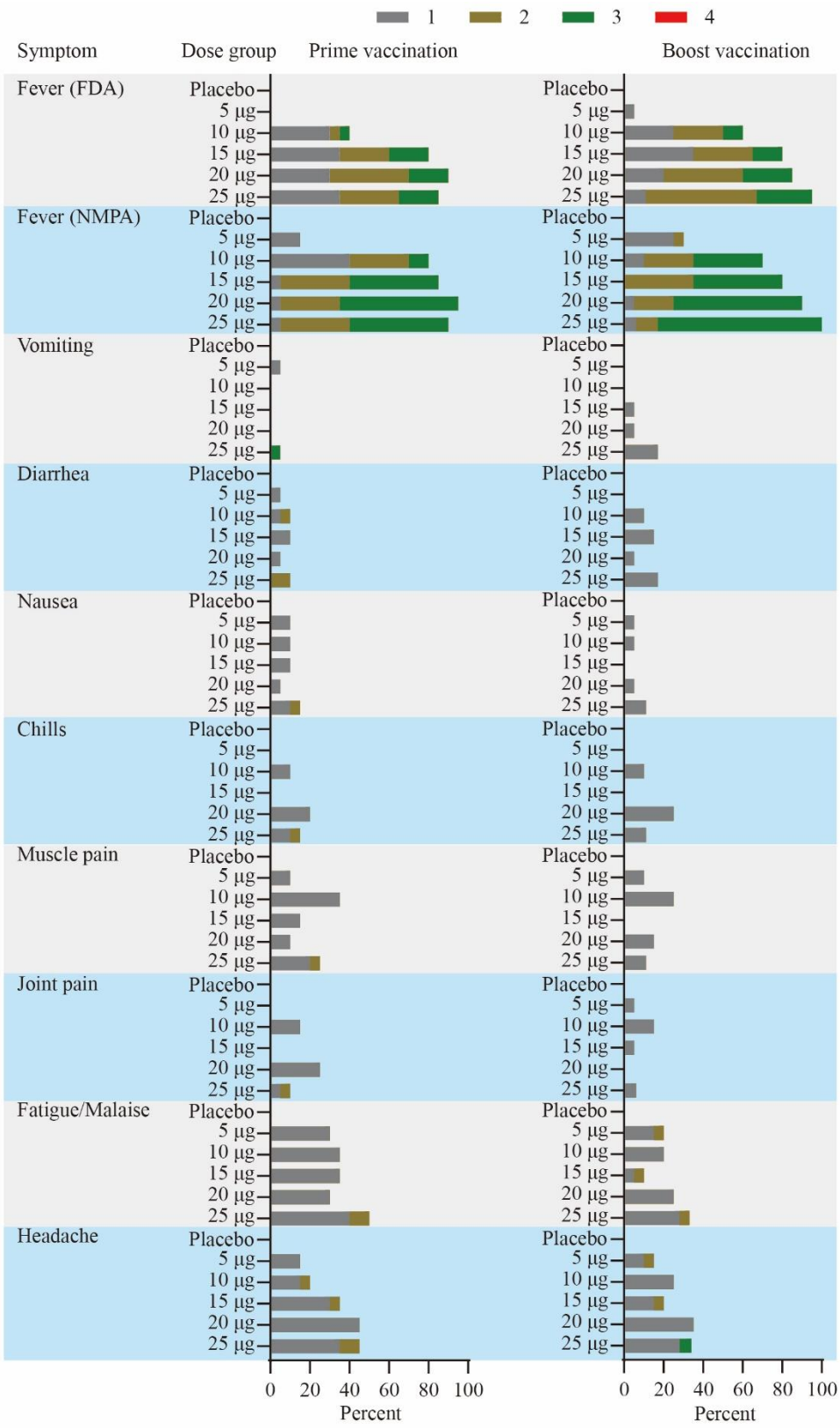

Figure 2-1: Systemic adverse events for 28 days after prime or boost vaccination in phase 1

Toxicity grading scales for systemic and local adverse events by NMPA and FDA are in the appendix 2 Table 2 and Table 3, respectively.

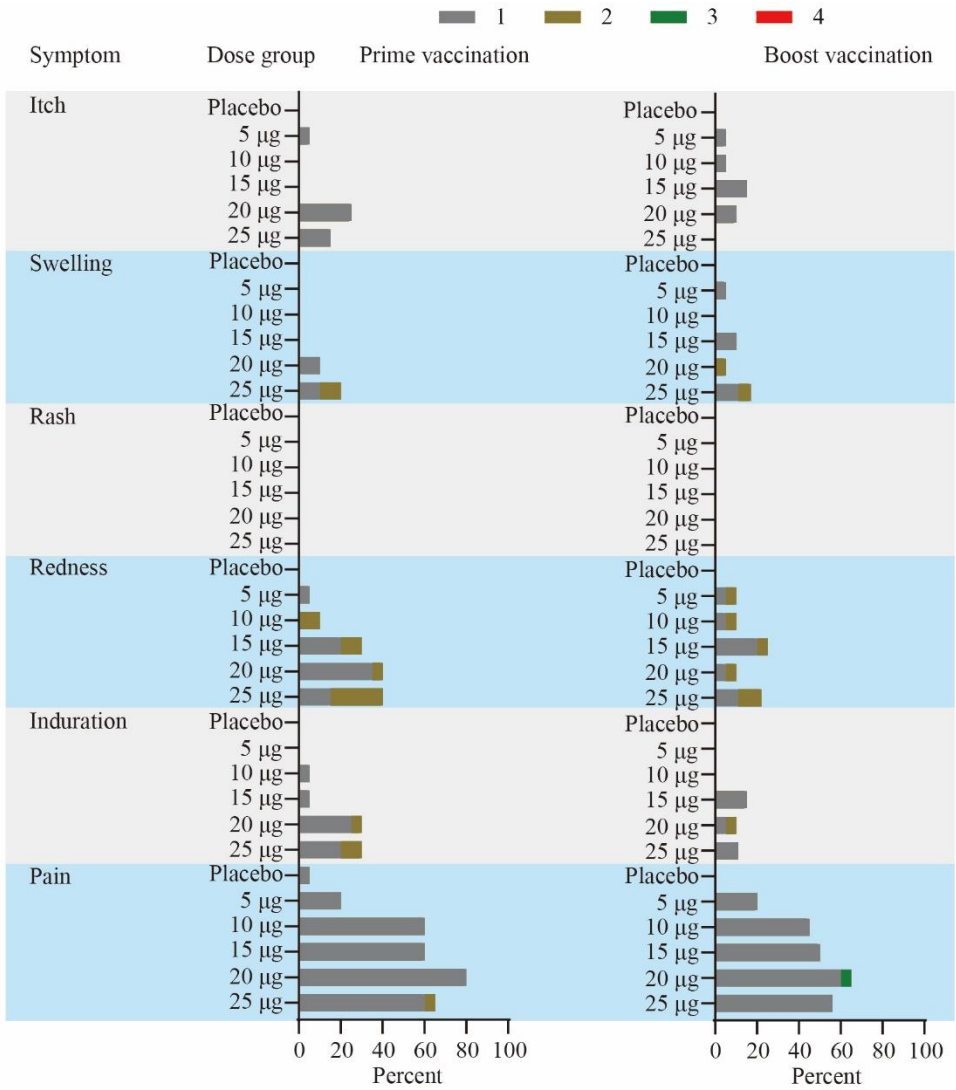

Figure 2-2: Local adverse events for 28 days after prime or boost vaccination in phase 1

Toxicity grading scales for systemic and local adverse events by NMPA and FDA are in the appendix 2 Table 2 and Table 3, respectively.

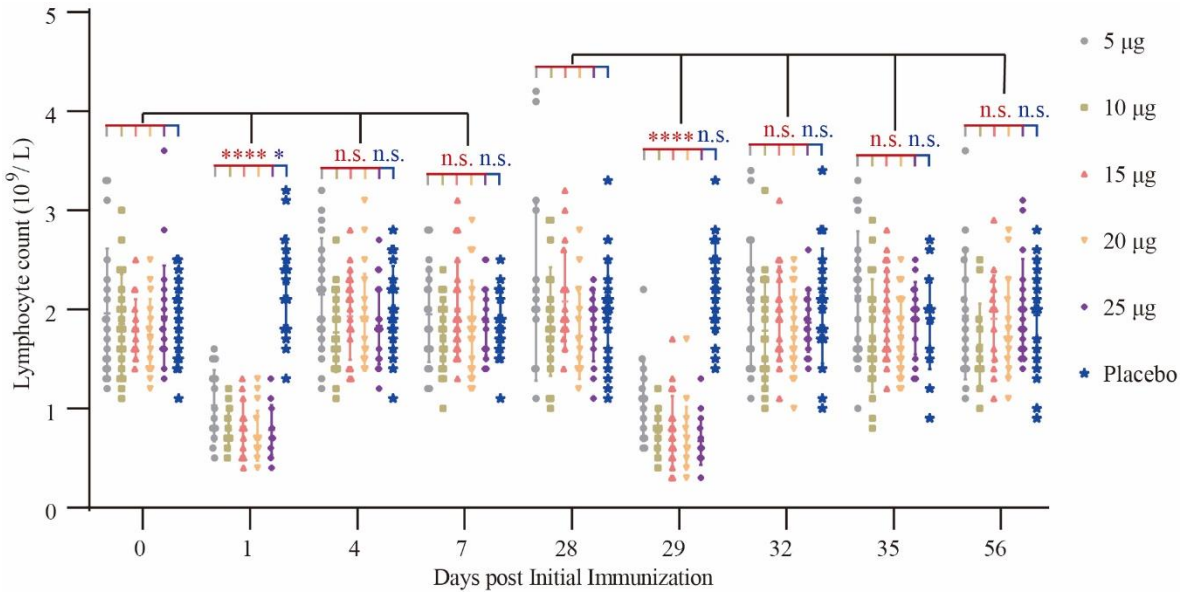

Figure 2-3: Lymphocyte counts in prime or boost vaccination in phase 1

Blood samples of the participants were collected at baseline (day 0), day 1, day 4, day 7, day 28 (before boost), day 29, day 32, day 35 and day 56 (after boost). The number of lymphocyte were counted. n.s. $p>0.05$, * $p<0.05$, **** $p<0.0001$.

Table 2-4: Blood biochemical indexes of the unsolicited adverse reactions for 14 days after prime or boost vaccinations graded by NMPA criteria in phase 1.

Blood biochemical indexes	5 μg (n=20)	10 μg (n=20)	15 μg (n=20)	20 μg (n=20)	25 μg (n=20)	placebo (n=16)	P value
ALT increased (U/L)	0	0	2 (10)	0	1 (5)	1 (6)	0.71
60 min	0	0	0	0	0	0	1.0
day 0-14	0	0	2 (10)	0	0	1 (6)	0.43
day 15-28	0	0	0	0	1 (5)	0	1.0
AST increased (U/L)	0	0	2 (10)	0	0	1 (6)	0.43
60 min	0	0	0	0	0	0	1.0
day 0-14	0	0	1 (5)	0	0	1 (6)	0.43
day 15-28	0	0	1 (5)	0	0	0	1.0
TBil increased (umol/L)	2 (10)	0	1 (5)	0	0	1 (6)	0.71
60 min	0	0	0	0	0	0	1.0
day 0-14	1 (5)	0	1 (5)	0	0	1 (6)	1.0
day 15-28	1 (5)	0	0	0	0	0	1.0
CK increased (U/L)	2 (10)	1 (5)	2 (10)	1 (5)	1 (5)	1 (6)	1.0
day 0-14	2 (10)	1 (5)	2 (10)	1 (5)	1 (5)	1 (6)	1.0
Cr (umol/L)	2 (10)	2 (10)	0	1 (5)	1 (5)	2 (13)	0.87
CRP increased (mg/L)	2 (10)	2 (10)	2 (10)	1 (5)	3 (15)	1 (6)	0.97
60 min	0	0	0	0	0	0	1.0000
day 0-14	2 (10)	1 (5)	2 (10)	1 (5)	3 (15)	1 (6)	0.93
day 15-28	0	1 (5)	0	0	0	0	1.0
LPS (U/L)	1 (5)	3 (15)	3 (15)	1 (5)	3 (15)	1 (6)	0.68
≥ Grade 3	0	1 (5)	0	0	0	1 (6)	1.0
60 min	0	0	0	0	0	0	1.0
day 0-14	1 (5)	1 (5)	2 (10)	1 (5)	3 (15)	1 (6)	0.90
day 15-28	0	3 (15)	1 (5)	0	0	0	0.087
Urea (umol/L)	0	0	0	0	0	0	1.0
Data are n (%)							

Note: ALT, Alamine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; CK: creatine kinase; Cr: Creatinine; CRP: C-reactive protein; LPS: Lipase

Participants withdrew

Table 3: Participants withdrew the trial

Reasons	Participants in group and quit time
Quit voluntarily	2 males in 25 μg group quit before boost dose 1 male in 25 μg group quit after boost dose
Received freeze-dried rabies vaccines for human use and tetanus vaccine due to cat scratch	1 female in 25 ug group quit after boost dose

Combined medication

Table 4-1: Combined medication after prime vaccination in phase 1

Medication	5 µg (n=20)	10 µg (n=20)	15 µg (n=20)	20 µg (n=20)	25 µg (n=20)	placebo (n=16)	P value
Total	1 (5)	6 (30)	16 (80)	17 (85)	16 (80)	3 (19)	<0.0001
Analgesics	0	6 (30)	16 (80)	17 (85)	16 (80)	1 (6)	<0.0001
Acetaminophen	0	6 (30)	16 (80)	17 (85)	16 (80)	0	<0.0001
Compound amphetamine	0	0	0	0	0	1 (6)	1.0
Ganmao Ling	0	0	0	1 (5)	0	0	1.0
Migranin	0	0	0	1 (5)	0	0	1.0
Others							
Celecoxib	0	0	4 (20)	5 (25)	4 (20)	0	0.0040
Polyene phosphatidyl choline	0	0	1 (5)	0	0	0	1.0
Glucose	0	0	0	0	1 (5)	0	1.0
Ebastine	0	0	0	0	0	1 (6)	1.0
Andrographolide	0	0	0	1 (5)	0	0	1.0
Amlodipine; atorvastatin calcium	1 (5)	0	0	0	0	0	1.0
Ibuprofen	0	0	0	0	0	1 (6)	1.0
Calamine	0	0	0	0	0	1 (6)	1.0

Data are n (%)

Table 4-2: Combined medication after boost vaccination in phase 1

Medication	5 μg (n=20)	10 μg (n=20)	15 μg (n=20)	20 μg (n=20)	25 μg (n=20)	placebo (n=18)	P value
Total	1 (5)	12 (60)	17 (85)	18 (90)	17 (94)	3 (15)	<0.0001
Analgesics	0	12 (60)	17 (85)	16 (80)	17 (94)	0	<0.0001
Acetaminophen	0	12 (60)	17 (85)	16 (80)	17 (94)	0	<0.0001
Dihydrocodeine tartrate; acetaminophen	0	0	1 (5)	0	0	0	1.0
Others							
Celecoxib	0	2 (10)	3 (15)	8 (40)	7 (39)	0	0.0001
Ibuprofen	0	0	1 (5)	0	0	0	1.0
Pudilan	0	0	0	1 (5)	0	0	1.0
Roxithromycin	0	0	0	2 (10)	0	0	0.16
Amoxicillin	0	0	0	1 (5)	0	0	1.0
Azithromycin	0	0	1 (5)	0	0	0	1.0
Cefaclor	0	0	0	0	1 (6)	0	0.15
Cefuroxime Axetil	0	0	0	1 (5)	0	0	1.0
Levofloxacin	0	1 (5)	0	0	0	0	1.0
Ganmao Qing	0	0	0	1 (5)	1 (6)	0	0.42
Acyclovir	0	0	0	0	0	1 (5)	1.0
Polyene phosphatidyl choline	0	0	0	0	0	1 (5)	1.0
Compound Glycyrrhizin	0	0	1 (5)	0	0	0	1.0
Fritillary bulb loquat	0	0	0	1 (5)	0	0	1.0
Glycyrrhiza	0	0	0	1 (5)	0	0	1.0
Strong loquat cream	0	0	1 (5)	0	0	0	1.0
Compound Licorice Flitilarry Bule and Ammonium Chloride	0	0	0	1 (5)	0	0	1.0
Qingkailing	0	1 (5)	0	0	0	0	1.0
Guilin watermelon frost	0	0	0	1 (5)	0	0	1.0
Betamethasone dipropionate; Betamethasone sodium phosphate	0	0	0	0	0	1 (5)	1.0
Hydrocortisone	0	0	0	0	0	1 (5)	1.0
Ebastine	0	0	0	0	0	1 (5)	1.0
Compound Sodium Chloride	1 (5)	0	0	0	0	0	1.0
Glucose and sodium chloride	1 (5)	0	0	0	0	0	1.0
Levofloxacin Hydrochloride	0	0	0	0	0	1 (5)	1.0
Compound Glycyrrhizin	0	0	0	0	0	1 (5)	1.0
Loratadine	0	0	0	0	0	1 (5)	1.0
Methoxamine; theophylline	0	0	0	1 (5)	0	0	1.0

Data are n (%)

Table 4-3: Combined non-medication after prime or boost vaccination in phase 1

Non-medication	5 μg (n=20)	10 μg (n=20)	15 μg (n=20)	20 μg (n=20)	25 μg (n=20)	placebo (n=18)	P value
Total	6 (30)	13 (65)	18 (90)	19 (95)	18 (90)	1 (5)	<0.0001
Surgical and medical procedures	6 (30)	13 (65)	18 (90)	19 (95)	18 (90)	1 (5)	<0.0001
Antipyresis	5 (25)	13 (65)	18 (90)	19 (95)	18 (90)	1 (5)	<0.0001
Fraction of inspired oxygen	1 (5)	0	0	0	0	0	1.0

Antibody response

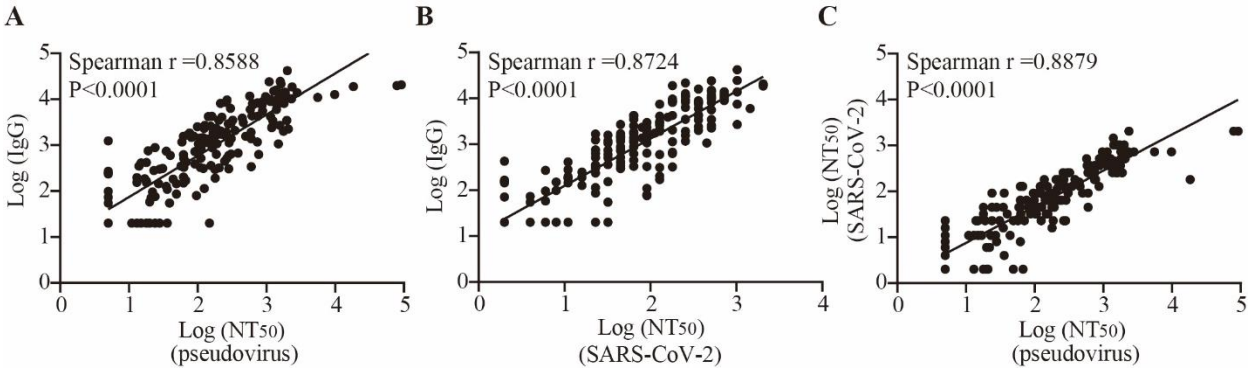

Figure 5: Correlation of IgG and neutralizing antibody titers

Correlation of RBD-specific IgG antibody and pseudovirus neutralizing antibody (A), RBD-specific IgG antibody and live SARS-CoV-2 neutralizing antibody (B), pseudovirus neutralizing antibody and live SARS-CoV-2 neutralizing antibody (C) of the vaccinated participants by day 43 and 56 are analyzed.

Methods

Table 6: Amino acid sequences of SARS-CoV-2 S-RBD peptides used in ELISpot assays

Peptide number	Amino acid start position*	Sequence
1	316	SNFRVQPTESIVRFP
2	321	QPTESIVRFPNITNL
3	326	IVRFPNITNLCPFGE
4	331	NITNLCPFGEVFNAT
5	336	CPFGEVFNATRFASV
6	341	VFNATRFASVYAWNR
7	346	RFASVYAWNRKRISN
8	351	YAWNRKRISNCVADY
9	356	KRISNCVADYSVLYN
10	361	CVADYSVLYNSASFS
11	366	SVLYNSASFSTFKCY
12	371	SASFSTFKCYGVSPT
13	376	TFKCYGVSPTKLNDL
14	381	GVSPTKLNDLCFTNV
15	386	KLNDLCFTNVYADSF
16	391	CFTNVYADSFVIRGD
17	396	YADSFVIRGDEVRQI
18	401	VIRGDEVRQIAPGQT
19	406	EVRQIAPGQTGKIAD
20	411	APGQTGKIADYNYKL
21	416	GKIADYNYKLPDDFT
22	421	YNYKLPDDFTGCVIA
23	426	PDDFTGCVIAWNSNN
24	431	GCVIAWNSNNLDSKV
25	436	WNSNNLDSKVGGNYN
26	441	LDSKVGGNYNYLYRL
27	446	GGNYNYLYRLFRKSN
28	451	YLYRLFRKSNLKPFE
29	456	FRKSNLKPFERDIST
30	461	LKPFERDISTEIYQA
31	466	RDISTEIYQAGSTPC
32	371	EIYQAGSTPCNGVEG
33	476	GSTPCNGVEGFNCYF
34	481	NGVEGFNCYFPLQSY
35	486	FNCYFPLQSYGFQPT
36	491	PLQSYGFQPTNGVGY
37	496	GFQPTNGVGYQPYRV
38	501	NGVGYQPYRVVVLSF
39	506	QPYRVVVLSFELLHA
40	511	VVLSFELLHAPATVC
41	516	ELLHAPATVCGPKKS
42	521	PATVCGPKKSTNLVK
43	526	GPKKSTNLVKNKCVN
44	531	TNLVKNKCVNFNFNG

*According to the SARS-CoV-2 spike protein (Gene ID: 43740568).

Methods

Standardized ELISA for IgG detection against SARS-CoV-2 S-RBD

SARS-CoV-2 RBD specific IgG titers were determined by a commercial ELISA kit (Beijing Wantai Biological) according to the manufacturer's instruction. Briefly, serial 2-fold dilutions of the subjects' serum, starting at 1:40, were added to blocked 96-well plates (50 μl/well) coated with recombinant SARS-CoV-2 RBD antigen and plates were incubated for 30 minutes at 37°C. After three washes with wash buffer, plates were added with Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (1: 5,000, ZSGB-BIO) or HRP-conjugated RBD and incubated for 30 minutes at 37 °C. Plates were then washed five times with wash buffer and added with chromogen solution followed by 15 minutes of incubation at 37 °C. The absorbance (450/630 nm) was read using a microplate reader (Bio Tek). The endpoint titers were defined according to the manufacturer's instruction.

Pseudovirus-based neutralization assay

In brief, Huh7 cells (JCRB, 0403) were seeded in 96-well plates (20,000 cells/well) and incubated for 20-28 h. Serial 3-fold diluted serum, starting at 1:10, were incubated with 650 TCID50 of the pseudovirus for 1 h at 37 °C. DMEM was used as negative control. The supernatant was then removed and luciferase substrate was added to each well followed by incubation for 2 minutes in darkness at room temperature. Luciferase activity was then measured using GloMax® 9633 Microplate Luminometer (Promega). The 50% neutralization titer (NT50) was defined as the serum dilution at which the relative light units (RLUs) were reduced by 50% compared with the virus control wells. The NT50 was determined by non-linear regression, i.e. log (inhibitor) *v.s.* normalized response (Variable slope), using GraphPad Prism 8.0 (GraphPad Software).

Microneutralization assay

SARS-CoV-2-specific neutralizing antibody titer in serum was determined using a CPE-based microneutralization assay with the SARS-CoV-2 virus strain BetaCoV/Beijing/IME-BJ01/2020 (Accession No. GWHACAX01000000) and Vero cells (ATCC, CCL-81). In brief, serum samples were heat-inactivated for 30 min at 56 °C and two fold serially diluted from 1:4 to 1: 2048 using DMEM (Thermo Fisher Scientific). Serum dilutions were then mixed with the same volume of virus solution to achieve a TCID 50 (50% tissue culture infectious dose) of 100 in each well. The serum-virus mixture was incubated at 37 °C for 1 h, then added to 96-well plates containing semi-confluent Vero cells (>80% density). After culturing at 37 °C for 3 d, CPEs on Vero cells were observed under an inverted microscope. The neutralizing titer is the reciprocal of the highest sample dilution that protects at least 50% of cells from CPE. If no neutralization reaction was observed at the initial serum dilution (1:4), an arbitrary titer of 2 (half of the limit of quantification) was reported.

Enzyme-linked immunospot (ELISpot) assay

Cellular immune responses in the vaccinated participants were assessed using IFN-γ, or IL-2 precoated ELISpot kits (MabTech), according to the manufacturer's protocol. Briefly, the plates were blocked using RPMI 1640 (Thermo Fisher Scientific) containing 10% AB serum and incubated for 30 minutes. The PBMCs of the participants were then plated at 250,000 cells/well, with peptide pool for SARS-CoV-2 RBD protein (2 μM of each peptide, see Appendix 6 Table 6), Anti-CD3 as positive control or RPMI 1640 media as negative control. After incubation at 37 °C, 5% CO2 for 48 h, plates were washed with wash buffer and 7-B6-ALP (for IFN-γ) or MT8G10-biotin (for IL-2) was added to each well followed by incubation for 2 h at room temperature. For IL-2, streptavidin-ALP was added after PBS washing and incubation for 1 h at room temperature. Following the addition of BCIP/NBT-plus substrate solution, the air-dried plates were read using the automated ELISpot reader AID ELISpot (AID). The numbers of spot-forming cells per 1,000,000 cells were calculated.

A Phase Ib Clinical Trial to Evaluate the Safety, Tolerability and Preliminary Immunogenicity of the SARS-CoV-2 mRNA Vaccine after Administered at Different Doses in Subjects Aged 18-59 Years

Generic Name of the Investigational Vaccine:	SARS-CoV-2 mRNA Vaccine
Phase:	Ib
Protocol No.	ARCoV-002
Version No.	V1.0
Last Revision Date of the Protocol:	September 28, 2020
Institution Responsible for the Clinical Study (seal):	Shulan (Hangzhou) Hospital
Sponsors (seal):	Academy of Military Medical Sciences Suzhou Abogen Biosciences Co., Ltd. Walvax Biotechnology Co., Ltd.

Signature Page of Sponsor

Protocol No.	ARCoV-002
Version No.	V1.0
Last Revision Date of the Protocol:	September 28, 2020
Study Title:	A Phase Ib Clinical Trial to Evaluate the Safety, Tolerability and Preliminary Immunogenicity of the SARS-CoV-2 mRNA Vaccine after Administered at Different Doses in Subjects Aged 18-59 Years
Sponsors:	Academy of Military Medical Sciences Address: 20#, East Avenue, Fengtai District, Beijing, China Post code: 100071 Responsible person: Chengfeng Qin Email: chengfeng_qin@126.com Suzhou Abogen Biosciences Co., Ltd. Address: B1-501, No. 218 Xinghu Street, Biobay, Suzhou, China Responsible person: Bo Ying Tel: +86 0512-69150735 Email: bo.ying@abogenbio.com Walvax Biotechnology Co., Ltd. Address: 3rd Floor, Building A3, Phase 2, Yunnan University Science & Technology Park, High & New Tech Development Zone, Kunming, Yunnan, China Post code: 650106 Responsible person: Runsheng Jiang Tel: +86 0871-68312889 Fax: +86 0871-68312567 E-mail: ynwsjrs@walvax.com Contact person of the sponsors: Shuyuan Yang Affiliation: Walvax Biotechnology Co., Ltd. Address: 3rd Floor, Building A3, Phase 2, Yunnan University Science & Technology Park, High & New Tech Development Zone, Kunming, Yunnan, China Post code: 650106 Tel: +86 18687832269 Fax: +86 0871-68312123 E-mail: ynwsysy@walvax.com
Approved by:	Lin Yuan Affiliation: Walvax Biotechnology Co.,Ltd. Address: 3rd Floor, Building A3, Phase 2, Yunnan University Science & Technology Park, High & New Tech Development Zone, Kunming, Yunnan, China Position: Technical director Signature: Date:

Signature Page of Principle Investigator

Protocol No.	ARCoV-002
Version No.	V1.0
Last Revision Date of the Protocol:	September 28, 2020
Study Title:	A Phase Ib Clinical Trial to Evaluate the Safety, Tolerability and Preliminary Immunogenicity of the SARS-CoV-2 mRNA Vaccine after Administered at Different Doses in Subjects Aged 18-59 Years

I hereby agree:

- To undertake the responsibility of correctly directing the conduct of this clinical study in the local area.
- To ensure that this clinical trial is conducted in accordance with the protocol and standard operating procedure of clinical studies.
- To ensure that the personnel involved in this project fully understand the information on the investigational vaccine as well as other study-related responsibilities and obligations specified in this study protocol.
- To ensure that no changes shall be made to the study protocol without the review and written approval from the sponsor and the Institutional Review Board (IRB), unless it is necessary to eliminate the immediate hazards to the subjects or to comply with the requirements of the regulatory authority (e.g., the administrative aspects of the project).
- I am fully familiar with the correct use of the investigational vaccine described in this study protocol, and fully understand other information provided, including but not limited to the following materials: current Investigator's Brochure (IB) or its equivalent, and supplement documents to the Investigator's Brochure (if any).
- I am familiar with and will comply with all current regulatory requirements in the *Good Clinical Practice* (GCP) and *Guidelines for Quality Management of Vaccine Clinical Trials* (for trial implementation).

Signature Page of Principle Investigator

Responsible Institution:	Shulan (Hangzhou) Hospital	
Principal Investigator	Lanjuan Li	Guiling Chen
Signature:		
Date:		

Signature Page of Principle Investigator

Protocol No.	ARCoV-002
Version No.	V1.0
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Signature Page of Principle Investigator

Responsible Institution: Guangxi Center for Disease Control and Prevention

Principal Investigator Zhaojun Mo

Signature:

Date:

List of Abbreviations

AE	Adverse Event
AEFI	Adverse Event Following Immunization
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
AVR	Adverse Vaccine Reaction
BMI	Body Mass Index
CD	Cluster of Differentiation
CDC	Center for Disease Control and Prevention
CNE	Cationic Nanoemulsion
CRE	Creatinine
CRF	Case Report Form
CRO	Contract Research Organization
DC	Dendritic Cell
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ELISA	Enzyme Linked Immunosorbent Assay
FIB	Fibrinogen
MedDRA	Medical Dictionary for Regulatory Activities
HIV	Human Immunodeficiency Virus
HGB	Hemoglobin
IFN	Interferon
IL	Interleukin
LNR	Lipid Nanoparticle
LP	Lipoplex
LPR	Lipopolyplex
LY	Lymphocyte
MONO	Monocytes
NEUT	Neutrophil
NMPA	National Medical Products Administration
PLT	Blood Platelet
PT	Prothrombin Time
RBC	Red Blood Cell
RBD	Receptor Binding Domain
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome-Coronavirus-2
Scr	Creatinine
SOP	Standard Operating Procedure
SS	Safety Set
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBIL	Total Bilirubin
TNF	Tumor Necrosis Factor
TT	Partial Thrombin Time
UREA	Urea
WBC	White Blood Cell
WHO	World Health Organization
MERS	Middle East Respiratory Syndrome

List of Terms

Adverse event: Any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participants' involvement in the research, whether or not considered related to participation in the research.

Serious adverse event: Any adverse event that results in death, life-threatening, or places the participant at immediate risk of death from the event as it occurred, requires or prolongs hospitalization, causes persistent or significant disability or incapacity, results in congenital anomalies or congenital disabilities, another condition which investigators judge to represent significant hazards.

Investigational drug: A drug with an active constituent or a placebo that be evaluated or used as a control drug in a clinical trial, including one that has been approved for marketing but for a different purpose than the approved content, for an unapproved indication, or for the purpose of obtaining more information about the approved content.

Audit: It refers to the systematic and independent inspection on clinical trial-related activities and documents so as to evaluate and determine whether the conduct of clinical trial-related activities and the recording, analysis and reporting of trial data meet the requirements prespecified in the study protocol, SOPs and relevant laws and regulations.

Subject: In the protocol, the recipients of a study product or control product, the investigator has contacted with themselves/ his or her parents/ guardian regarding to participation in the clinical study.

Solicited adverse event: Adverse events collected in clinical studies as the end points of clinical studies refer to the information of adverse events actively collected by subjects or observers during a specific follow-up period after vaccination, i.e., presence/occurrence/severity.

Unsolicited adverse event: Adverse events other than solicitated adverse events reported in clinical studies. It also includes solicited symptoms reported beyond the specified solicitation time.

Investigator Brochure: The Investigator's Brochure (IB) is a compilation of the clinical and nonclinical data on the investigational product(s) that are relevant to the study of the product(s) in human subjects.

Synopsis

<table>
<tr><td>Study Name</td><td>A Phase Ib Clinical Trial to Evaluate the Safety, Tolerability and Preliminary Immunogenicity of the SARS-CoV-2 mRNA Vaccine after Administered at Different Doses in Subjects Aged 18-59 Years</td></tr>
<tr><td>Product Characteristics</td><td>The SARS-CoV-2 mRNA Vaccine is formulated by encapsulating the mRNA, which encodes the receptor-binding domain (RBD) of spike glycoprotein (S protein) of SARS-CoV-2 and is transcribed in-vitro by the corresponding DNA template, in lipid nanoparticles (LNPs). The length of the mRNA fragment is 1080nt and the theoretical molecular weight is 347kDa. The vaccine is clear colorless liquid.</td></tr>
<tr><td>Indications</td><td>This vaccine is indicated for active immunization in healthy people against COVID-19 caused by the SARS-CoV-2 infection.</td></tr>
<tr><td>Study Population</td><td>Healthy people aged 18-59 years in China.</td></tr>
<tr><td>Number of Subjects</td><td>120</td></tr>
<tr><td>Brief Introduction of the Study</td><td>Since December 2019, a COVID-19 outbreak has occurred in Wuhan, Hubei. With the spread of the epidemic, many other cities in China and other countries have also found such cases. As an acute respiratory infectious disease, the disease has been included in the class B infectious disease stipulated in the Law of the People's Republic of China on Prevention and Treatment of Infectious Diseases, and is managed as class A infectious diseases. Through a series of prevention, control and medical treatment measures, the rising trend of the epidemic situation in China has been curbed to a certain extent, and the epidemic situation in most provinces has been alleviated, but the number of cases outside the country is still on the rise.

SARS-CoV-2 mRNA vaccine (ARCoV) has been granted the Drug Clinical Trial Approval approved by the National Medical Products Administration (NMPA) on June 19, 2020, and started the Phase I clinical trial (ARCoV-001) in Shulan (Hangzhou) Hospital on June 29, 2020. Up to now, the preliminary safety and immunogenicity results of the 25 μg group have been generated in the phase I clinical trial, which provides a data reference for continuing the clinical research of other dosage of this product.

This is a phase Ib clinical trial of a SARS-CoV-2 mRNA vaccine (ARCoV-002). The trial will be conducted with a single-center, randomized, double-blind, placebo-controlled design. Subjects aged 18-59 will be vaccinated with different dosages (5 μg / 10 μg / 15 μg / 20 μg / 25 μg) or placebo (normal saline) according to the 2-dose immunization schedule (Days 0 and 28). To evaluate the safety, tolerability and preliminary immunogenicity of different doses of the vaccine according to the 0-and 28-day immunization schedule. At the same time, according to the preliminary safety and immunogenicity results, the immune persistence of the recommended dosage group will be explored in order to provide reference for the follow-up clinical trials of the vaccine.</td></tr>
<tr><td>Experimental Vaccine</td><td>Study vaccine: SARS-CoV-2 mRNA vaccine (ARCoV)

Manufacturer: Suzhou Abogen Biosciences Co., Ltd.

Specification and composition:
<table>
<tr><td>Dose</td><td>Specification</td><td>Contents of mRNA</td></tr>
<tr><td>5 μg</td><td>0.5mL/vial</td><td>5 μg</td></tr>
<tr><td>10 μg</td><td>0.5mL/vial</td><td>10 μg</td></tr>
<tr><td>15 μg</td><td>0.5mL/vial</td><td>15 μg</td></tr>
<tr><td>20 μg</td><td>0.5mL/vial</td><td>20 μg</td></tr>
<tr><td>25 μg</td><td>0.5mL/vial</td><td>25 μg</td></tr>
</table></td></tr>
</table>

<table>
<tr><td></td><td>
Description: clear colorless liquid
Batch No. and expiration date:
<table>
<tr><th>Dose</th><th>Batch Number</th><th>Expiration Date</th></tr>
<tr><td>5 μg</td><td>AE2008001</td><td>Aug 11, 2022</td></tr>
<tr><td>10 μg</td><td>AD2008001</td><td>Aug 10, 2022</td></tr>
<tr><td>15 μg</td><td>AC2008001</td><td>Aug 09, 2022</td></tr>
<tr><td>20 μg</td><td>AB2008001</td><td>Aug 12, 2022</td></tr>
<tr><td>25 μg</td><td>AA2008001</td><td>Aug 08, 2022</td></tr>
</table>
Administration route: intramuscular injection into deltoid muscle of the lateral upper arm.

Immunization schedule: administer 1 dose each on Day 0 and 28 respectively, 2 doses in total.

Storage and transportation conditions: store and transport at 2-8℃ with protection from light. Strictly prevent from freezing.

Placebo: normal saline

Manufacturer: Suzhou Abogen Biosciences Co., Ltd.

Specification and composition: 0.5 ml/vial, 0.9% sodium chloride solution.

Description: clear colorless liquid.

Batch No. and expiration date: D2005001, February 2022.

Administration route: intramuscular injection in the deltoid muscle of lateral upper arm.

Immunization schedule: administer 1 dose each on Day 0 and 28 respectively, 2 doses in total.

Storage and transportation conditions: should be stored and transported away from light under 2-8℃ and strictly prevented from freezing.
</td></tr>
<tr><td>Study Objectives</td><td>
1. To evaluate the safety, tolerability, and preliminary immunogenicity of the investigational vaccine after administered at different doses in subjects aged 18-59.
2. To explore the immune persistence and specific cellular immune response to the RBD segment of S protein induced by the investigational vaccine at the recommended dose
</td></tr>
<tr><td>Study Endpoints</td><td>
Primary Endpoints:

The incidence rates of adverse reactions/events observed within 60 minutes and 0-14 days after each dose, as well as those observed within 0-28 days after each dose.

Secondary Endpoints:

➢ Secondary Safety Endpoints
1. Incidence rates of adverse reactions or events regarding measurements of blood biochemistry, hematology, coagulation, and urinalysis at 1, 4, 7 and 28 days after each dose in all subjects.
2. Incidence rates of serious adverse events observed from Dose 1 to 12 months after complete series in all subjects.

➢ Secondary Immunogenicity Endpoints
1. Levels of neutralizing antibodies against SARS-CoV-2 as measured by pseudovirus neutralization test at 28 days after Dose 1 as well as 7, 15 and 28 days after complete series in all subjects.
</td></tr>
</table>

	2. Levels of neutralizing antibodies against SARS-CoV-2 as measured by wild-type virus neutralization test at 28 days after Dose 1 and 7, 15 and 28 days after complete series in all subjects. 3. Levels of specific IgG antibodies against SARS-CoV-2 at 28 days after Dose 1 as well as 7, 14 and 28 days after complete series in all subjects. **Exploratory endpoints:** ➢ **Exploratory Safety Endpoints** 1. The incidence rate of SARS-CoV-2 infections and the incidence rates of severe and critical cases observed from Dose 1 to 12 months after complete series in all subjects. 2. Incidence rates of autoimmune diseases observed from Dose 1 of administration to 12 months after complete series in all subjects. 3. Incidence rates of tumors observed from Dose 1 to 12 months after complete series in all subjects. 4. Pregnancy outcomes, delivery characteristics, delivery conditions, as well as growth and development of neonates within 12 months after birth (including pregnancy in female subjects and that in the spouse of male subjects) observed from Dose 1 to 12 months after complete series in all subjects. ➢ **Exploratory Immunogenicity Endpoints** ***Humoral immunity (for subjects in the recommended-dose study group):*** 1. Levels of neutralizing antibodies against SARS-CoV-2 as measured by pseudovirus neutralization test at 3 months, 6 months, 9 months, and 12 months after complete series in subjects included in the recommended-dose study group. 2. Levels of neutralizing antibodies against SARS-CoV-2 as measured by wild-type virus neutralization test at 3 months, 6 months, 9 months, and 12 months after complete series in subjects included in the recommended-dose study group. 3. Levels of specific IgG antibodies against SARS-CoV-2 at 3 months, 6 months, 9 months, and 12 months after complete series in subjects included in the recommended-dose study group. ***Cell-mediated immunity (for the CMI subgroup, the first 12 subjects in each dose group will be included in the CMI subgroup):*** 1. The proportions of cell subsets including $CD4^+$, $CD8^+$, $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}2^+$, $CD4^+TNF\alpha^+$, $CD4^+IL\text{-}4^+$, $CD4^+IL\text{-}13^+$, $CD8^+IFN\text{-}\gamma^+$, $CD8^+IL\text{-}2^+$, and $CD8^+TNF\alpha^+$ specific to Spike protein RBD (as measured by flow cytometry) at 7 days after Dose 1, 7 days and 15 days after the complete series in subjects included in the specific cellular immunogenicity subgroup, as well as those measured at 6 months after complete series in subjects included in the specific cellular immunogenicity subgroup of the recommended-dose study group; 2. Levels of cytokines including IFN-γ and IL-2 specific to Spike protein RBD (as measured by ELISpot) at 7 days after Dose 1, 7 days, and 15 days after the complete series in subjects included in the specific cellular immunogenicity subgroup, as well as those measured at 6 months after complete series in subjects included in the specific cellular immunogenicity subgroup of the recommended-dose study group. 3. The proportions of memory T cells including $CCR7^+CD45RA^-CD4^+$ and $CCR7^+CD45RA^-CD8^+$ specific to Spike protein RBD at 28 days after complete series in subjects included in the specific cellular immunogenicity subgroup as well as those measured at 3 and 6 months after complete series in subjects included in the specific cellular immunogenicity subgroup of the

	recommended-dose study group. In this trial, the exploratory endpoints of specific CMI may be adjusted according to the results generated from the early-stage clinical trial. **Halting rules or termination of the study:** ➢ **Halting rules for Sentinels** During the safety observation period after Dose 1, if any of the following halting rules are triggered, a DSMB meeting will be held for safety assessment: 1) Vaccine-related death or life-threatening serious adverse events occur in any of the sentinels in the corresponding dose group. 2) Vaccine-related ulceration, abscess or necrosis at the injection site occur in any of the sentinels in the corresponding dose group. 3) Severe acute allergic reactions such as laryngospasm, tracheospasm, systemic edema combined with hypotension or gastrointestinal spasm occurs in any of the sentinels in the corresponding dose group within 24 hours after administration. 4) Systemic urticaria occurs in any of the sentinels in the corresponding dose group within 72 hours after administration (systemic reaction is defined as having 3 or more sites of the body observed with the symptom). 5) Any sentinel in the corresponding dose group experiences a fever with a body temperature > 40 ℃ within 7 days after administration. 6) More than 2 sentinels in the corresponding dose group experience an adverse event with a severity grade of 3 or above, lasting for > 48 hours without remission (remission: the adverse event is resolved after reasonable interventions* administered by investigators or its severity reduces to below grade 3 without any intervention), and is possibly/definitely related to vaccination or without reasonable attribution to causes other than vaccination. If a dose group triggers the halting rule for sentinels, all subjects in that group and groups of higher doses should be halted for subsequent study procedures. ➢ **Halting rules for the Study:** In case of any of the following situation occurs during the 28-day safety observation, a DSMB meeting shall be held to review whether to continue the study: 1) Vaccine-related death or life-threatening serious adverse events occur in any of the subjects in the corresponding dose group. 2) Ulceration, abscess, or necrosis at the injection site related to vaccination occur in any subject in the corresponding dose group. 3) Severe acute allergic reactions such as laryngospasm, tracheospasm, systemic edema combined with hypotension or gastrointestinal spasm occurs within 24 hours after vaccination in any subject in corresponding dose group. 4) Systemic urticaria occurs in ≥ 3 subjects in the corresponding dose group within 72 hours after vaccination (systemic reaction is defined as having 3 or more sites of the body observed with the symptom). 5) Any subject in the corresponding dose group experiences a fever with a body temperature > 40 ℃ within 7 days after vaccination. 6) After vaccination, the subject in the corresponding dose group experiences an adverse event with a severity grade of 3 or above, lasting for > 48 hours without remission (remission: the adverse event is resolved after reasonable

	interventions* administered by investigators or its severity reduces to below grade 3 without any intervention), and is possibly/definitely related to vaccination or without reasonable attribution to causes other than vaccination. If a dose group triggers the halting rules for the study, all subjects in that group and groups of higher doses should be halted for subsequent study procedures. *:"reasonable interventions" means the relevant measures specified in the Risk Control Plan for ADR.
Study Design	**Study Design** This study adopts a single-center, randomized, double-blind and placebo-controlled design. **Sample Size and Grouping** According to the requirements prescribed in the Technical Guidelines for Clinical Trials of Vaccines, a phase I clinical trial is a small-scale study including 20-30 subjects, focusing on confirming the clinical tolerance and safety. A total of 120 subjects aged 18-59 years are to be enrolled in this study, with 24 subjects in each group, who will be randomized into the study group (n = 20) or the control group (n = 4) in a ratio of 5:1. All subjects will be vaccinated with the investigational vaccine at corresponding dose or placebo (normal saline) 28 days apart. The grouping, sample size and study number of the subjects are shown in the following table:

Group	Dose	Sample Size		Study No.
		Study Group	Control Group	
A	5 μg	20	4	001-024
B	10 μg	20	4	025-048
C	15 μg	20	4	049-072
D	20 μg	20	4	073-096
E	25 μg	20	4	097-120

Specific cell-mediated immunogenicity subgroup:

According to the order of study No. (ascending), the first 12 subjects in each group will be included in the specific cell immunogenicity subgroup (n = 60), in which the exploratory endpoints of specific CMI will be detected.

Study Procedures:

1. By ascending order of doses, the six sentinels in the preceding dose group will be given the first dose of the investigational vaccine or placebo, and the preliminary safety observation will be performed for 4 days post administration. If the halting rules for sentinels are not met, the other 18 subjects in the same group will be enrolled, and the following dose group will start rolling sentinels at the same time. Otherwise, the preliminary safety data obtained from 6 sentinels will be submitted to DSMB for review. Whether the remaining 18 subjects of this dose group as well as the sentinels in the following dose group should be enrolled will be decided according to the opinions from the DSMB meeting.
2. Dose 2 will be administered following Dose 1 as planned if the specified halting rules are not triggered.
3. A routine DSMB meeting will be convened upon all subjects have completed the 14-day safety observation post Dose 2 to evaluate the available safety data of each group and propose on whether the trial could be proceeded to phase

II.

4. A routine DSMB meeting will be convened upon all subjects have completed the 28-day safety observation post Dose 2 to evaluate the overall 28-day safety post complete series in the phase Ib clinical trial.
5. If any group triggers the halting rules for sentinels or for the study, the group and the groups of higher doses will be interrupted. A provisional DSMB meeting will be convened to evaluate the safety of the above-mentioned groups. Whether the study procedure will continue for these groups will be discussed and determined on the recommendations from DSMB.

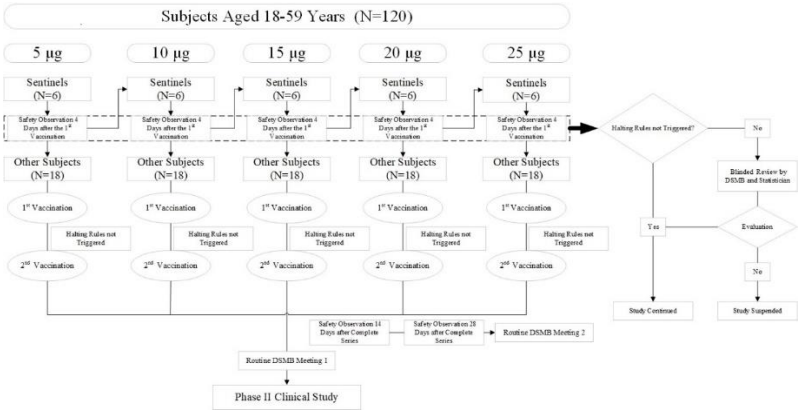

Figure 1 Phase Ib Clinical Trial Process

Sentinels: when each group enrolls subjects, the first 6 subjects will be "sentinels". All sentinels will be admitted to hospital before Dose 1 and will be hospitalized for 24 hours (1 day) after Dose 1. Based on the observation results on the first day of hospitalization, the investigators shall determine whether the length of hospitalization is to be extended for further observation or the sentinels might leave for home for passive monitoring. Based on the safety observations of the sentinels after Dose 1, the investigators shall determine whether hospitalization is needed for sentinels after Dose 2 and for the remaining subjects in the same group post vaccination.

Preliminary evaluation of phase Ib clinical trial: after the completion of 14-day safety observation post complete series for all subjects in each group, a routine DSMB meeting will be convened to evaluate the available data of each group and propose recommendations on whether the trial could be proceeded into phase II. After the completion of 28-day observation following complete series in all subjects in each dose group, a routine DSMB meeting will be convened to evaluate the overall 28-day safety post complete series for the phase Ib clinical trial.

DSMB: DSMB meetings are either routine or provisional. The DSMB proposal will be provided after discussion in the DSMB meeting, after which the sponsor and the investigator will jointly decide whether to continue the study. For detailed DSMB regulations, please refer to the *Regulations of Independent Data and Safety Monitoring Board for Phase Ib Clinical Trials*.

Routine DSMB meeting: 1. Upon all subjects have completed the 14-day safety observation after complete series; 2. Upon all subjects have completed the 28-day safety observation after complete series.

Provisional DSMB meeting: in case that any halting rule for sentinels or for study is triggered during the study process, DSMB meeting shall be held to review the data as to determine whether to continue the trial.

Safety Observation:

- **Safety observation from Dose 1 to 0-28 days after complete series**

	All adverse events observed within 60 minutes after each dose, as well as the injection-site (local) and non-injection-site (systemic) adverse events observed during 0-14 days after each dose will be collected from all subjects. Unsolicited adverse events will be collected during 0-28 days after each dose. Blood biochemistry, hematology, blood coagulation test and urinalysis will be performed before each dose, as well as at 1, 4, 7 and 28 days after each dose for all subjects. ➢ **Long-term safety observation** The following measurements will be collected from all subjects through Dose 1 to 12 months post complete series: all serious adverse events; the occurrence of SARS-CoV-2 infections and the incidence rates of severe or critical cases; the occurrence of autoimmune diseases or tumors; pregnancy related events (pregnancy outcomes, delivery characteristics, neonatal delivery of the female subjects or the spouse of male subjects, and the growth and development of neonates). **Immunogenicity Observation:** ➢ **Immunogenicity observation from Dose 1 to 28 days after complete series** Venous blood samples will be collected before Dose 1, 28 days after Dose 1 as well as 7, 15 and 28 days after complete series for humoral immunity evaluation. Measurements to be tested include levels of neutralizing antibodies against SARS-CoV-2 as measured by pseudovirus neutralization test, levels of neutralizing antibodies against SARS-CoV-2 as measured by wild-type virus neutralization test and levels of specific IgG antibodies. ➢ **Immune persistence observation** According to the preliminary statistical analysis results for immunogenicity from Dose 1 to 28 days after complete series, taking into account the immunogenicity and safety profile of each dose group comprehensively, the immune persistence will be investigated in the recommended-dose study group. Blood samples of subjects in the recommended-dose study group will be collected at 3, 6, 9 and 12 months after complete series, and the humoral immunity (levels of neutralizing antibodies against SARS-CoV-2 as measured by pseudovirus/wild type virus neutralization test and specific IgG antibodies) at corresponding time point will be detected. ➢ **Exploratory observation of CMI** 1 The venous blood samples will be collected from subjects in the specific CMI subgroup before Dose 1, 7 days after Dose 1, as well as at 7 and 15 days after complete series, respectively to detect the proportions of cell subsets including $CD4^+$, $CD8^+$, $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}2^+$, $CD4^+TNF\alpha^+$, $CD4^+IL\text{-}4^+$, $CD4^+IL\text{-}13^+$, $CD8^+IFN\text{-}\gamma^+$, $CD8^+IL\text{-}2^+$, and $CD8^+TNF\alpha^+$ specific to Spike protein RBD (as measured by flow cytometry). 2 The venous blood samples will be collected from subjects in the specific CMI subgroup before Dose 1, 7 days after Dose 1, as well as at 7 and 15 days after complete series, respectively to detect the levels of cytokines including IFN-γ and IL-2 specific to Spike protein RBD (ELISpot). 3 The venous blood samples will be collected from subjects in the specific CMI subgroup 28 days after complete series to detect the proportions of memory T cells including $CCR7^+CD45RA\text{-}CD4^+$ and $CCR7^+CD45RA\text{-}CD8^+$ (as measured by flow cytometry). 4 The venous blood will also be collected from subjects in the specific CMI

	subgroup of the recommended-dose group at 3 and 6 months post complete series to detect the proportions of above-mentioned specific cell subsets and the levels of cytokines after complete series (as measured by flow cytometry and ELISpot performed at the 6th month), as well as the proportions of memory T cells (as measured by flow cytometry performed at the 3rd and 6th months after complete series). The exploratory observation of CMI in this trial may be adjusted according to the observations of early-stage clinical trial. **Follow-up Plan:** ➢ **Subjects in the specific CMI subgroup (including "Sentinels")** Sentinels (hospitalized for 1 day after Dose 1): a total of 11 on-site visits will be required within 28 days after complete series to complete enrollment screening (Visit 0), two doses of vaccination (Visit 1, 6), laboratory investigations after immunization (Visit 2-4, 6-9, 11), collection of adverse events (Visit 1-11), humoral immunity observation (Visit 6, 9-11) and specific CMI observation (Visit 4, 9-11). Visit 2 can be completed during hospitalization. Subjects in the specific CMI subgroup: a total of 12 on-site visits will be required within 28 days after complete series to complete enrollment screening (Visit 0), two doses of vaccination (Visit 1, 6), laboratory investigations after immunization (Visit 2-4, 6-9, 11), collection of adverse events (Visit 1-11), humoral immunity observation (Visit 6, 9-11) and specific CMI observation (Visit 4, 9-11). In addition to the above-mentioned on-site visits, additional 14 telephone interviews will be required: (1) once at 4-6 hours, 2 days, 3 days, and 5 days after each dose, respectively (8 times in total), to collect the occurrence of adverse events (telephone visits for sentinels can be exempted during hospitalization); (2) once every 2 months after complete series (6 times in total) to collect the occurrence of serious adverse events, SARS-CoV-2 infections, severe critical cases, autoimmune diseases or tumors, and pregnancy-related events (pregnancy outcomes delivery characteristics, delivery of neonates as well as growth and development within 12 months after birth). In addition to the above-mentioned on-site and telephone visits, investigators can increase the number of visits or telephone interviews as appropriate, and compile corresponding follow-up records. ➢ **Other subjects** A total of 12 on-site visits will be needed within 28 days after complete series to complete the enrollment screening (Visit 0), two doses of vaccination (Visit 1 and Visit 6), laboratory investigations after immunization (Visit 2-4, 6-9, 11), collection of adverse reactions (Visit 1-11) as well as humoral immunity observation (Visit 6, 9-11). In addition to the above-mentioned visits, another 14 telephone interviews will be needed for subjects included in the specific CMI subgroup (including "Sentinel" subjects), as described in the section “Subjects in the specific CMI subgroup (including "Sentinel" subjects)”. ➢ **Subjects in the recommended-dose group (including the subjects in the specific CMI subgroup of the recommended-dose group)** The schedule of on-site visits within 28 days after complete series is the same to that described in “Subjects in the specific CMI subgroup (including "Sentinel" subjects)” and “Other subjects”. A total of 4 on-site visits (Visit 12-15) will be conducted 28 days after complete series to complete blood collection for humoral immunity of immune persistence

	observation. For subjects included in the specific CMI subgroup in the recommended-dose group, additional blood samples will be collected at 3 and 6 months after complete series (Visit 12-13) to complete the observation of exploratory endpoints for specific CMI. In addition to the above-mentioned on-site visits, 14 telephone visits will also be needed, as described in the "Subjects in the specific CMI subgroup (including "Sentinel" subjects)". When the scheduled time of on-site visits coincides with the prescribed time of telephone interviews, the visits can be fused into one. **Data Collection:** The Electronic Data Capture (EDC) system will be used to collect necessary data for statistical analysis.
Inclusion Criteria	Subjects are required to meet all of the following inclusion criteria: 1. Healthy adults aged 18-59 years and are able to provide legal identity certificate. Both male and female should be included. 2. The subjects shall fully understand the content of the informed consent form and the characteristics of the study vaccine, voluntarily sign the informed consent form, as well as have the ability to use thermometer, scale and fill in diary card and contact card as required. 3. The subjects shall be able to communicate well with the investigators, as well as understand and comply with the requirements of this study. 4. The subjects' axillary temperature on the day of administration should be less than 37.3℃.
Exclusion Criteria	Subjects who meet any of the following exclusion criteria should be excluded from the study: 1. Individuals whose results from a complete physical examination do not meet the healthy standards, mainly include: • Abnormal vital signs with clinical significance (pulse < 50/min or > 100/min, systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg). • BMI <18 kg/m^2 or >30 kg/m^2. • In the screening window, the laboratory test value exceeds 1.2 times of the upper limit or lower limit of the normal reference range, and is determined by the investigator to be clinically significant. 2. Women with positive results of pregnancy test (those who are in menstrual period or undergo amenorrhea for at least 1 year or is surgically sterilized with evidenced medical records can be exempted from pregnancy test), or women in lactation period, or women who plan to conceive from screening period to 12 months after complete series, men whose spouse plans to conceive, or those who plan to donate sperm and eggs. 3. Women in menstrual period (from the 1st day to the 4th day of menstrual cycle). 4. Individuals with history of vaccination with SARS-CoV-2 vaccines. 5. Individuals with prior diagnosis as COVID-19 cases or suspected cases. Individuals who have a history of contact with COVID-19 cases or suspected cases within 1 month before signing the informed consent, or those who have overseas travel history or have traveled to areas with high incidence of COVID-19; COVID-19 infector or carriers: serum SARS-CoV-2 specific antibody or SARS-CoV-2 nucleic acid is tested to be positive in throat swabs. 6. Those who are tested positive for hepatitis B surface antigen, hepatitis C core antigen, hepatitis C virus antibody, treponema pallidum specific antibody and human immunodeficiency virus antibody. 7. Those who have a history of SARS, MERS and other coronavirus infections or diseases in the past.

	8. Those who have acute diseases or acute attack of chronic diseases within 2 weeks prior to the first dose of vaccination, or have fever (axillary temperature ≥ 37.3℃) or upper respiratory tract infection within 7 days prior to the first dose of vaccination. 9. Those who have a history of severe side effects induced by any vaccine or drug, such as allergy, urticaria, eczema, dyspnea, angioneurotic edema, etc. 10. Those who have been vaccinated with any vaccine within 1 month prior to Dose 1. 11. Those who cannot tolerate the venipuncture and have fainting history during acupuncture or at the sight of blood. 12. Those who have hereditary bleeding tendency or coagulation dysfunction, or have history of thrombosis or bleeding, and the detection results of coagulation function related measures are abnormal. 13. Those who have been diagnosed with congenital or acquired immunodeficiency (e.g., HIV infection). 14. Those who have asplenia or functional asplenia, or have other important organs removed by surgery for any reason. 15. Those who previously have serious diseases with abnormal clinical manifestations that needed to be excluded, including but not limited to a history of systemic diseases of nervous system, cardiovascular system, blood and lymphatic system, immune system, kidney, liver, gastrointestinal tract, respiratory system, metabolic and skeletal system, and malignant neoplasms (excluding controllable and stable chronic diseases such as diabetes, hypertension, etc.). 16. Those who have undergone surgery within 3 months prior to the signing of informed consent, or who plan to have surgery during the trial period or within 3 months after the end of the trial (including cosmetic surgery, dental surgery, and oral surgery). 17. Those who had blood donation or blood loss (≥450 mL), blood transfusion or use of blood products within 3 months prior to the signing of the informed consent, or plan to donate blood during the trial period. 18. Those who used any investigational or unlicensed products (drug, vaccine, biological product, or device) other than the study vaccine within 3 months prior to the signing of the informed consent, or who plan to use the above-mentioned products during the study period. 19. Those who received immunosuppressive therapy within 6 months prior to signing the informed consent, such as long-term systemic glucocorticoid therapy (more than 2 weeks of continuous systemic glucocorticoid therapy, such as prednisone or similar drugs, within 6 months). Topical application (such as ointment, eye drops, inhalants, or nasal sprays) is allowed. Local medication should not exceed the dosage recommended in the prescribing information and the subjects should not have any signs of systemic exposure. 20. Those who are determined by the investigator to be not suitable to participate in the trial.
Exclusion Criteria for Dose 2	If any of the following conditions occur during the administration, vaccination for subjects should be postponed or terminated, but other study procedures can be continued according to the discretion of the investigator. Dose 2 should be administered 28 days after Dose 1 with a window of + 5 days: 1. Those who with positive results of urine pregnancy test. 2. For women in menstrual period (from the 1st day to the 4th day of menstrual cycle), Dose 2 should be postponed to the 5th day (inclusive) of the menstrual cycle 3. Those who develop serious allergic reactions or serious adverse events with

	causal relationship with vaccination after Dose 1. 4. Those who are administered with any vaccine other than the study vaccine after Dose 1. 5. Those who have close contact with COVID-19 cases after Dose 1. 6. Those who are determined by the investigator to be not suitable to participate in the trial.
Criteria for Early Termination of Study	1. If the subject develops disability, life-threatening and other adverse events or serious adverse events, the investigator shall determine if the subject's participation in the study should be terminated according to the need for treatment. 2. The subjects voluntarily ask to withdraw from the clinical study, or their health status does not allow them to continue to participate in the study. 3. 3) Other situations where the investigator determines the subjects not suitable to continue participating in the study.
Study Hypothesis	Not applicable.
Relevant Definition	1. 18-59 years old: 18 years old or older (i.e., on the day of 18 years old), but no more than 60 years old (i.e., the day before 60 years old). Calculation method of one year old: on the birthday of the Gregorian calendar, the age is increased by one year. For example, subjects born on January 1, 2000 will be 20 years old on January 1, 2020, and so on. 2. One month is defined as 30 days.

Study Team

Protocol No.	ARCoV-002
Version No.	V1.0
Version Date	September 28, 2020
Study Title	A Phase Ib Clinical Trial to Evaluate the Safety, Tolerability and Preliminary Immunogenicity of the SARS-CoV-2 mRNA Vaccine after Administered at Different Doses in Subjects Aged 18-59 Years

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All parties of the clinical trial shall follow: (1) relevant regulations in the *Vaccine Administration Law of the People's Republic of China, Good Clinical Practice (GCP)* and *Guidelines for Good Clinical Practice for Vaccine Clinical Trials (For Trial Implementation)*; (2) Technical service contracts and confidentiality agreements signed by the sponsor and all parties in the clinical trial; (3) Relevant provisions in Module 13. Quality Assurance and Monitoring of Clinical Studies in this protocol. Refer to Annex 2 of this protocol for a brief description of the study facility.

Table of Contents

1 Study Title

A Phase Ib Clinical Trial to Evaluate the Safety, Tolerability and Preliminary Immunogenicity of the SARS-CoV-2 mRNA Vaccine after Administered at Different Doses in Subjects Aged 18-59 Years

2 Introduction

Since December 2019, a COVID-19 outbreak has occurred in Wuhan, Hubei. With the spread of the epidemic, many other cities in China and other countries have also found such cases. As an acute respiratory infectious disease, the disease has been included in the class B infectious disease stipulated in *the Law of the People's Republic of China on Prevention and Treatment of Infectious Diseases*, and is managed as class A infectious diseases. Through a series of prevention, control and medical treatment measures, the rising trend of the epidemic situation in China has been curbed to a certain extent, and the epidemic situation in most provinces has been alleviated, but the number of cases outside the country is still on the rise.

SARS-CoV-2 mRNA vaccine has been granted the *Drug Clinical Trial Approval* approved by the National Medical Products Administration (NMPA) on June 19, 2020, and started the Phase I clinical trial (ARCoV-001) in Shulan (Hangzhou) Hospital on June 29, 2020. Up to now, the preliminary safety and immunogenicity results of the 25 μg group have been generated in the phase I clinical trial, which provides a data reference for continuing the clinical research of other dosage of this product.

This is a phase Ib clinical trial (ARCoV-002) of a SARS-CoV-2 mRNA vaccine (ARCoV). The trial will be conducted with a single-center, randomized, double-blind, placebo-controlled design. Subjects aged 18-59 will be vaccinated with different dosages (5 μg / 10 μg / 15 μg / 20 μg / 25 μg) or placebo (normal saline) according to the 2-dose immunization schedule (Days 0 and 28). To evaluate the safety, tolerability, and preliminary immunogenicity of different doses of the vaccine according to the 0-and 28-day immunization schedule. At the same time, according to the preliminary safety and immunogenicity results, the immune persistence of the recommended dosage group will be explored in order to provide reference for the follow-up clinical trials of the vaccine.

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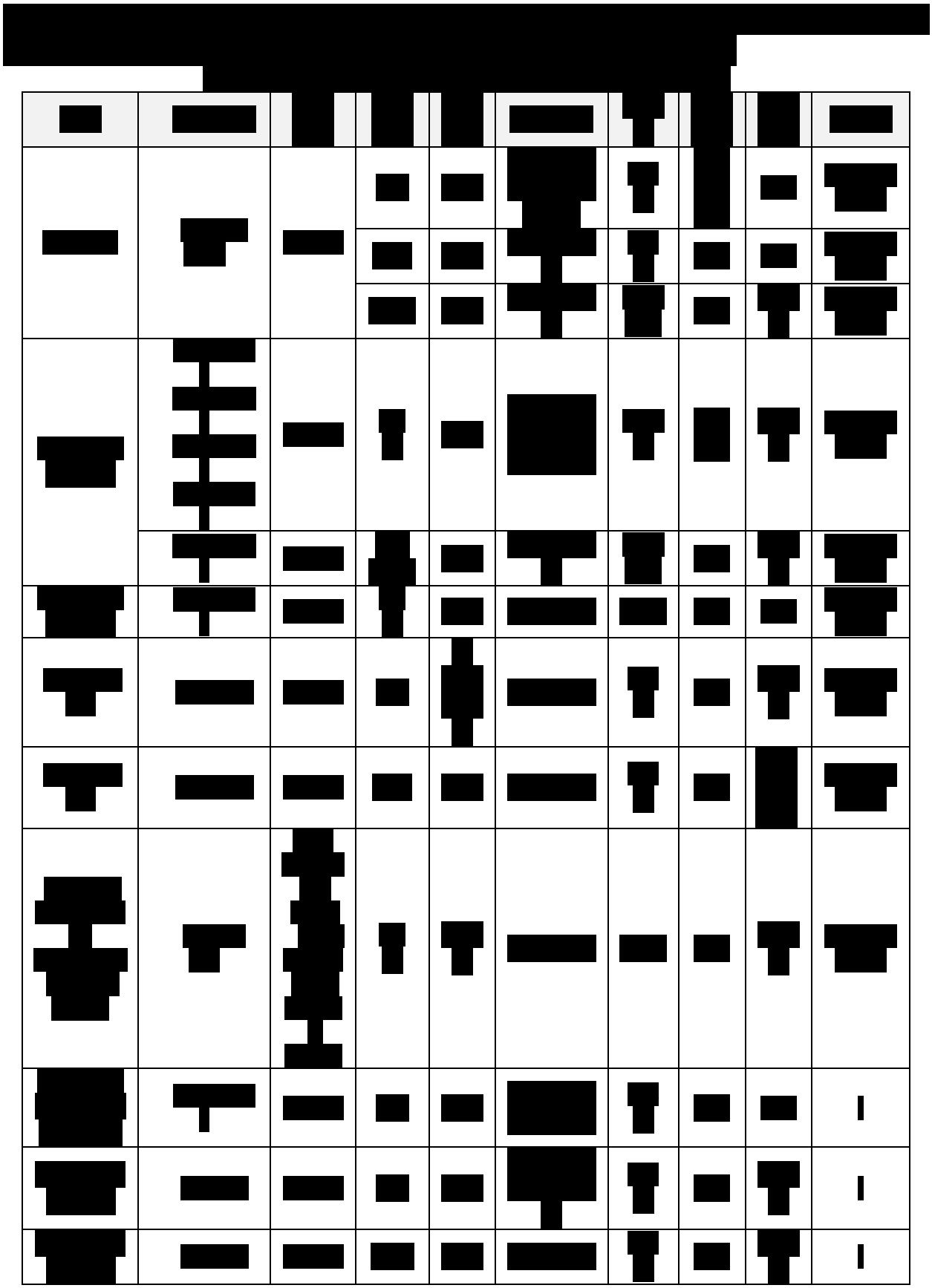

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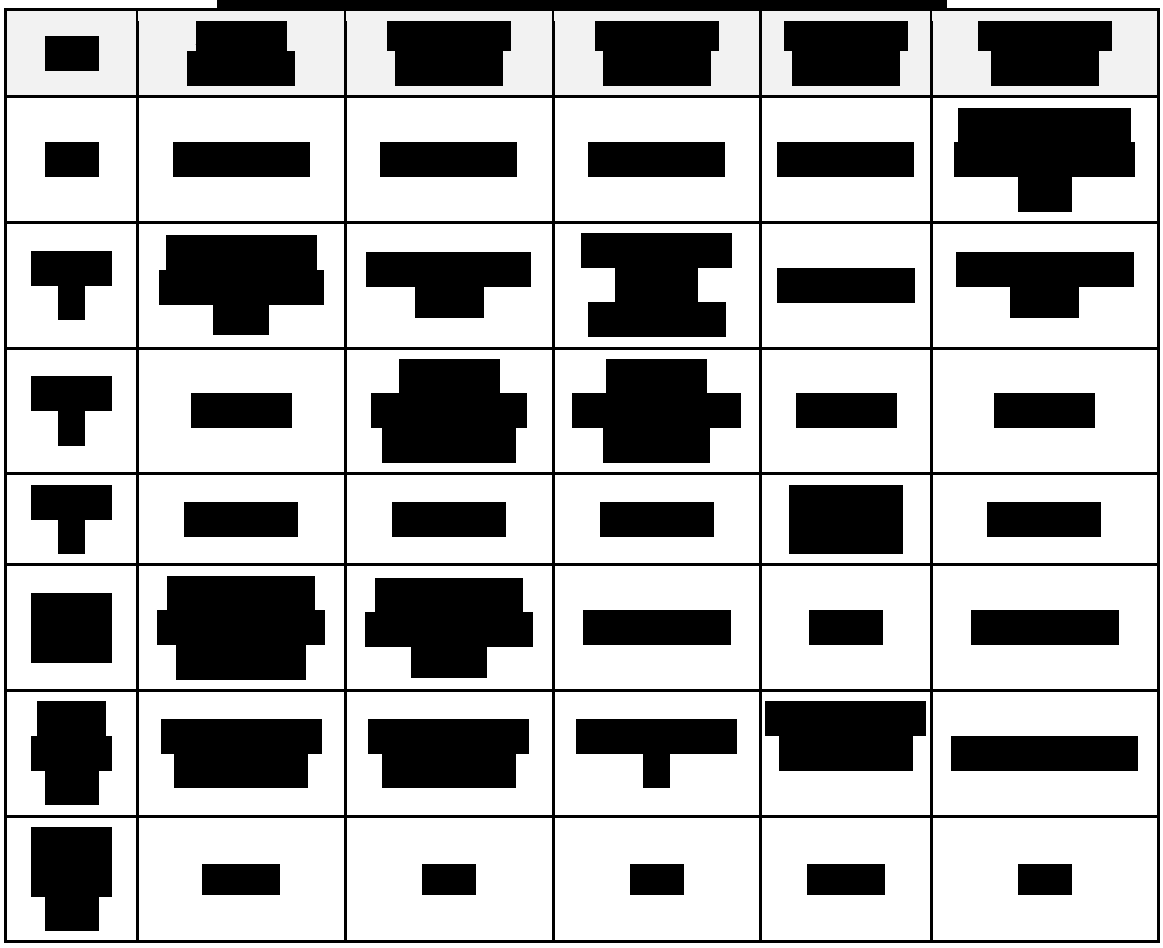

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5 Study Population

The subjects in this trial are healthy individuals aged 18-59 in China.

6 Study Design

6.1 Study Objectives

1. To evaluate the safety, tolerability, and preliminary immunogenicity of the investigational vaccine after administered at different doses in subjects aged 18-59.
2. To explore the immune persistence and specific cellular immune response to the RBD segment of S protein induced by the investigational vaccine at the recommended dose.

6.2 Study Endpoints

6.2.1 Primary Endpoints

The incidence rates of adverse reactions/events observed within 60 minutes and 0-14 days after each dose, as well as those observed within 0-28 days after each dose.

6.2.2 Secondary Endpoints

- **Secondary Safety Endpoints**

3. Incidence rates of adverse reactions or events regarding measurements of blood biochemistry, hematology, coagulation, and urinalysis at 1, 4, 7 and 28 days after each dose in all subjects.
4. Incidence rates of serious adverse events observed from Dose 1 to 12 months after complete series in all subjects.

- **Secondary Immunogenicity Endpoints**

4. Levels of neutralizing antibodies against SARS-CoV-2 as measured by pseudovirus neutralization test at 28 days after Dose 1 as well as 7, 15 and 28 days after complete series in all subjects.
5. Levels of neutralizing antibodies against SARS-CoV-2 as measured by wild-type virus neutralization test at 28 days after Dose 1 and 7, 15 and 28 days after complete series in all subjects.
6. Levels of specific IgG antibodies against SARS-CoV-2 at 28 days after Dose 1 as well as 7, 14 and 28 days after complete series in all subjects.

6.2.3 Exploratory Endpoints

- **Exploratory Safety Endpoints**

5. The incidence rate of SARS-CoV-2 infections and the incidence rates of severe and critical cases observed from Dose 1 to 12 months after complete series in all subjects.
6. Incidence rates of autoimmune diseases observed from Dose 1 of administration to 12 months after complete series in all subjects.
7. Incidence rates of tumors observed from Dose 1 to 12 months after complete series in all subjects.
8. Pregnancy outcomes, delivery characteristics, delivery conditions, as well as growth and development of neonates within 12 months after birth (including pregnancy in female subjects and that in the spouse of male subjects) observed from Dose 1 to 12 months after complete series in all subjects.

- **Exploratory Immunogenicity Endpoints**

Humoral immunity (for subjects in the recommended-dose study group):

4. Levels of neutralizing antibodies against SARS-CoV-2 as measured by pseudovirus neutralization test at 3 months, 6 months, 9 months, and 12 months after complete series in subjects included in the recommended-dose study group.
5. Levels of neutralizing antibodies against SARS-CoV-2 as measured by wild-type virus neutralization test at 3 months, 6 months, 9 months, and 12 months after complete series in subjects included in the recommended-dose study group.
6. Levels of specific IgG antibodies against SARS-CoV-2 at 3 months, 6 months, 9 months, and 12 months after complete series in subjects included in the recommended-dose study group.

Cell-mediated immunity (for the CMI subgroup, the first 12 subjects in each dose group will be included in the CMI subgroup):

4. The proportions of cell subsets including $CD4^+$, $CD8^+$, $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}2^+$, $CD4^+TNF\alpha^+$, $CD4^+IL\text{-}4^+$, $CD4^+IL\text{-}13^+$, $CD8^+IFN\text{-}\gamma^+$, $CD8^+IL\text{-}2^+$, and $CD8^+TNF\alpha^+$ specific to Spike protein RBD (as measured by flow cytometry) at 7 days after Dose 1, 7 days and 15 days after the complete series in subjects included in the specific cellular immunogenicity subgroup, as well as those measured at 6 months after complete series in subjects included in the specific cellular immunogenicity subgroup of the recommended-dose study group;
5. Levels of cytokines including IFN-γ and IL-2 specific to Spike protein RBD (as measured by ELISpot) at 7 days after Dose 1, 7 days, and 15 days after the complete series in subjects included in the specific cellular immunogenicity subgroup, as well as those measured at 6 months after complete series in subjects included in the specific cellular immunogenicity subgroup of the recommended-dose study group.
6. The proportions of memory T cells including $CCR7^+CD45RA^-CD4^+$ and $CCR7^+CD45RA^-CD8^+$ specific to Spike protein RBD at 28 days after complete series in subjects included in the specific cellular immunogenicity subgroup as well as those measured at 3 and 6 months after complete series in subjects included in the specific cellular immunogenicity subgroup of the recommended-dose study group.

In this trial, the exploratory endpoints of specific CMI may be adjusted according to the results generated from the early-stage clinical trial.

6.3 Study Design

This study adopts a single-center, randomized, double-blind and placebo-controlled design.

6.4 Sample Size and Grouping

According to the requirements prescribed in the *Technical Guidelines for Clinical Trials of Vaccines,* a phase I clinical trial is a small-scale study including 20-30 subjects, focusing on confirming the clinical tolerance and safety.

A total of 120 subjects aged 18-59 years are to be enrolled in this study, with 24 subjects in each group, who will be randomized into the study group (n = 20) or the control group (n = 4) in a ratio of 5:1. All subjects will be vaccinated with the investigational vaccine at corresponding dose or placebo (normal saline) 28 days apart. The grouping, sample size and study number of the subjects are shown in the following table:

Group	Dose	Sample Size		Study No.
		Study Group	Control Group	
A	5 μg	20	4	001-024

B	10 μg	20	4	025-048
C	15 μg	20	4	049-072
D	20 μg	20	4	073-096
E	25 μg	20	4	097-120

Specific cell-mediated immunogenicity subgroup:

According to the order of study No. (ascending), the first 12 subjects in each group will be included in the specific cell immunogenicity subgroup (n = 60), in which the exploratory endpoints of specific CMI will be detected.

6.5 Study Procedures

1) By ascending order of doses, the six sentinels in the preceding dose group will be given the first dose of the investigational vaccine or placebo, and the preliminary safety observation will be performed for 4 days post administration. If the halting rules for sentinels are not met, the other 18 subjects in the same group will be enrolled, and the following dose group will start rolling sentinels at the same time. Otherwise, the preliminary safety data obtained from 6 sentinels will be submitted to DSMB for review. Whether the remaining 18 subjects of this dose group as well as the sentinels in the following dose group should be enrolled will be decided according to the opinions from the DSMB meeting.

2) Dose 2 will be administered following Dose 1 as planned if the specified halting rules are not triggered.

3) A routine DSMB meeting will be convened upon all subjects have completed the 14-day safety observation post Dose 2 to evaluate the available safety data of each group and propose on whether the trial could be proceeded to phase II.

4) A routine DSMB meeting will be convened upon all subjects have completed the 28-day safety observation post Dose 2 to evaluate the overall 28-day safety post complete series in the phase Ib clinical trial.

5) If any group triggers the halting rules for sentinels or for the study, the group and the groups of higher doses will be interrupted. A provisional DSMB meeting will be convened to evaluate the safety of the above-mentioned groups. Whether the study procedure will continue for these groups will be discussed and determined on the recommendations from DSMB.

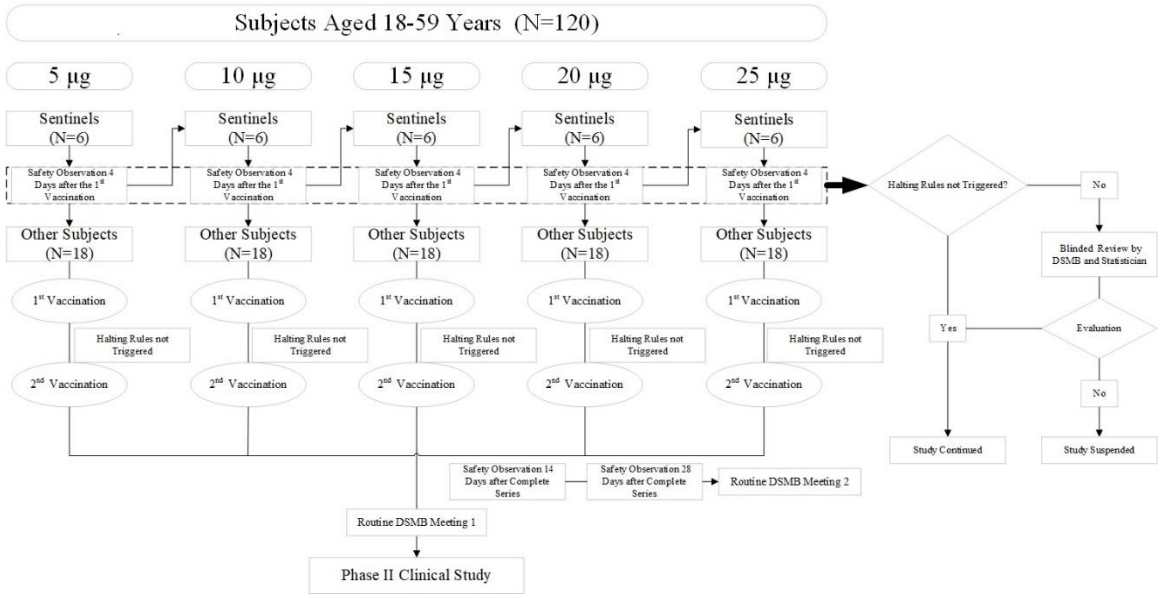

Figure 2 Phase Ib Clinical Trial Process

Sentinels: when each group enrolls subjects, the first 6 subjects will be "sentinels". All sentinels will be admitted to hospital before Dose 1 and will be hospitalized for 24 hours (1 day) after Dose 1. Based on the observation results on the first day of hospitalization, the investigators shall determine whether the length of hospitalization is to be extended for further observation or the sentinels might leave for home for passive monitoring. Based on the safety observations of the sentinels after Dose 1, the investigators shall determine whether hospitalization is needed for sentinels after Dose 2 and for the remaining subjects in the same group post vaccination.

Preliminary evaluation of the phase Ib clinical trial: after the completion of 14-day safety observation post complete series for all subjects in each group, a routine DSMB meeting will be convened to evaluate the available data of each group and propose recommendations on whether the trial could be proceeded into phase II. After the completion of 28-day observation following complete series in all subjects in each dose group, a routine DSMB meeting will be convened to evaluate the overall 28-day safety post complete series for the phase Ib clinical trial.

DSMB: DSMB meetings are either routine or provisional. The DSMB proposal will be provided after discussion in the DSMB meeting, after which the sponsor and the investigator will jointly decide whether to continue the study. For detailed DSMB regulations, please refer to the *Regulations of Independent Data and Safety Monitoring Board for Phase Ib Clinical Trials*.

Routine DSMB meeting: 1. Upon all subjects have completed the 14-day safety observation after complete series; 2. Upon all subjects have completed the 28-day safety observation after complete series.

Provisional DSMB meeting: in case that any halting rule for sentinels or for study is triggered during the study process, DSMB meeting shall be held to review the data as to determine whether to continue the trial.

6.6 Safety Observation

- **Safety observation from Dose 1 to 0-28 days after complete series**

All adverse events observed within 60 minutes after each dose, as well as the injection-site (local) and non-injection-site (systemic) adverse events observed during 0-14 days after each dose will be collected from all subjects. Unsolicited adverse events will be collected during 0-28 days after each dose.

Blood biochemistry, hematology, blood coagulation test and urinalysis will be performed before each dose, as well as at 1, 4, 7 and 28 days after each dose for all subjects.

- **Long-term safety observation**

The following measurements will be collected from all subjects through Dose 1 to 12 months post complete series: all serious adverse events; the occurrence of SARS-CoV-2 infections and the incidence rates of severe or critical cases; the occurrence of autoimmune diseases or tumors; pregnancy related events (pregnancy outcomes, delivery characteristics, neonatal delivery of the female subjects or the spouse of male subjects, and the growth and development of neonates).

6.7 Immunogenicity Observation

- **Immunogenicity observation from Dose 1 to 28 days after complete series**

Venous blood samples will be collected before Dose 1, 28 days after Dose 1 as well as 7, 15 and 28 days after complete series for humoral immunity evaluation. Measurements to be tested include levels of neutralizing antibodies against SARS-CoV-2 as measured by pseudovirus

neutralization test, levels of neutralizing antibodies against SARS-CoV-2 as measured by wild-type virus neutralization test and levels of specific IgG antibodies.

- **Immune persistence observation**

According to the preliminary statistical analysis results for immunogenicity from Dose 1 to 28 days after complete series, taking into account the immunogenicity and safety profile of each dose group comprehensively, the immune persistence will be investigated in the recommended-dose study group.

Blood samples of subjects in the recommended-dose study group will be collected at 3, 6, 9 and 12 months after complete series, and the humoral immunity (levels of neutralizing antibodies against SARS-CoV-2 as measured by pseudovirus/wild type virus neutralization test and specific IgG antibodies) at corresponding time point will be detected.

- **Exploratory observation of CMI**

1. The venous blood samples will be collected from subjects in the specific CMI subgroup before Dose 1, 7 days after Dose 1, as well as at 7 and 15 days after complete series, respectively to detect the proportions of cell subsets including $CD4^+$, $CD8^+$, $CD4^+$IFN-γ^+, $CD4^+$IL-2^+, $CD4^+TNF\alpha^+$, $CD4^+$IL-4^+, $CD4^+$IL-13^+, $CD8^+$IFN-γ^+, $CD8^+$IL-2^+, and $CD8^+TNF\alpha^+$ specific to Spike protein RBD (as measured by flow cytometry).
2. The venous blood samples will be collected from subjects in the specific CMI subgroup before Dose 1, 7 days after Dose 1, as well as at 7 and 15 days after complete series, respectively to detect the levels of cytokines including IFN-γ and IL-2 specific to Spike protein RBD (ELISpot).
3. The venous blood samples will be collected from subjects in the specific CMI subgroup 28 days after complete series to detect the proportions of memory T cells including $CCR7^+CD45RA$-$CD4^+$ and $CCR7^+CD45RA$-$CD8^+$ (as measured by flow cytometry).
4. The venous blood will also be collected from subjects in the specific CMI subgroup of the recommended-dose group at 3 and 6 months post complete series to detect the proportions of above-mentioned specific cell subsets and the levels of cytokines after complete series (as measured by flow cytometry and ELISpot performed at the 6^{th} month), as well as the proportions of memory T cells (as measured by flow cytometry performed at the 3^{rd} and 6^{th} months after complete series).

The exploratory observation of CMI in this trial may be adjusted according to the observations of early-stage clinical trial.

6.8 Follow-up Plan

- **Subjects in the specific CMI subgroup (including "Sentinel" subjects)**

Sentinels (hospitalized for 1 day after Dose 1): a total of 11 on-site visits will be required within 28 days after complete series to complete enrollment screening (Visit 0), two doses of vaccination (Visit 1, 6), laboratory investigations after immunization (Visit 2-4, 6-9, 11), collection of adverse events (Visit 1-11), humoral immunity observation (Visit 6, 9-11) and specific CMI observation (Visit 4, 9-11). Visit 2 can be completed during hospitalization.

Subjects in the specific CMI subgroup: a total of 12 on-site visits will be required within 28 days after complete series to complete enrollment screening (Visit 0), two doses of vaccination (Visit 1, 6), laboratory investigations after immunization (Visit 2-4, 6-9, 11), collection of adverse events (Visit 1-11), humoral immunity observation (Visit 6, 9-11) and specific CMI observation (Visit 4, 9-11).

In addition to the above-mentioned on-site visits, additional 14 telephone interviews will be required: (1) once at 4-6 hours, 2 days, 3 days, and 5 days after each dose, respectively (8 times in total), to collect the occurrence of adverse events (telephone visits for sentinels can be exempted during hospitalization); (2) once every 2 months after complete series (6 times in total) to collect the occurrence of serious adverse events, SARS-CoV-2 infections, severe critical cases, autoimmune diseases or tumors, and pregnancy-related events (pregnancy outcomes delivery characteristics, delivery of neonates as well as growth and development within 12 months after birth).

In addition to the above-mentioned on-site and telephone visits, investigators can increase the number of visits or telephone interviews as appropriate, and compile corresponding follow-up records.

- **Other subjects**

A total of 12 on-site visits will be needed within 28 days after complete series to complete the enrollment screening (Visit 0), two doses of vaccination (Visit 1 and Visit 6), laboratory investigations after immunization (Visit 2-4, 6-9, 11), collection of adverse reactions (Visit 1-11) as well as humoral immunity observation (Visit 6, 9-11).

In addition to the above-mentioned visits, another 14 telephone interviews will be needed for subjects included in the specific CMI subgroup (including "Sentinel" subjects), as described in the section “Subjects in the specific CMI subgroup (including "Sentinel" subjects)”.

- **Subjects in the recommended-dose group (including the subjects in the specific CMI subgroup of the recommended-dose group)**

The schedule of on-site visits within 28 days after complete series is the same to that described in “Subjects in the specific CMI subgroup (including "Sentinel" subjects)” and “Other subjects”.

A total of 4 on-site visits (Visit 12-15) will be conducted 28 days after complete series to complete blood collection for humoral immunity of immune persistence observation. For subjects included in the specific CMI subgroup in the recommended-dose group, additional blood samples will be collected at 3 and 6 months after complete series (Visit 12-13) to complete the observation of exploratory endpoints for specific CMI.

In addition to the above-mentioned on-site visits, 14 telephone visits will also be needed, as described in the “Subjects in the specific CMI subgroup (including "Sentinel" subjects)”. When the scheduled time of on-site visits coincides with the prescribed time of telephone interviews, the visits can be fused into one.

6.9 Data Collection

The Electronic Data Capture (EDC) system will be used to collect necessary data for statistical analysis.

6.10 Randomization

In this trial, the experimental vaccines will be sequentially administered at doses of 5 μg/10 μg/15 μg/20 μg/25 μg. Subjects in each dose group will be randomly administered with the investigational vaccine or placebo at corresponding doses. The first 6 enrolled subjects in each dose group will serve as sentinels, with 5 of them receiving the investigational vaccine and one receiving the placebo in a 5:1 ratio. After the safety is verified, the remaining 18 subjects will be randomized in a 5:1 ratio, of which 15 subjects will receive the investigational vaccine and 3 will receive the placebo.

6.10.1 Randomization

The randomized blinding codes will be generated by the randomization statistician using the block randomization method through SAS statistical software (version 9.4). The investigator will assign a randomized study number to the subject after verifying that the subject has signed the informed consent form and has been screened to be eligible for inclusion. The study staff will administer the vaccine of the same number as the study number to the subject. For randomized subjects who withdraw from the clinical trial for any reason, the study number will be retained regardless of whether or not the investigational vaccine has been administered and the assigned study number cannot be re-assigned.

Study numbers of subjects in each dose group:

(1) The study number range of 5 μg dose group is 001 ~ 024.

(2) The study number range of 10 μg dose group is 025 ~ 048.

(3) The study number range of 15 μg dose group is 049 ~ 072.

(4) The study number range of 20 μg dose group is 073 ~ 096.

(5) The study number range of 25 μg dose group is 097~120.

The first 12 subjects by study number aged 18-59 years in each dose group will be included in the specific cellular immunogenicity subgroup.

Backup vaccine: The block randomization method will be used to generate a backup vaccine randomization table. The number of backup vaccines will be 60 in total (10 vials of the investigational vaccine at each dose of 5 μg/10 μg/15 μg/20 μg/25 μg and 10 vials of the placebo). In case that the backup vaccines are needed during the study, the investigator will log in to the backup vaccine acquisition system to obtain the corresponding backup vaccine number.

6.10.2 Blinding

This study will be designed as double-blind. After all subjects complete the 28-day visits post complete series, the subjects, investigators, sponsors, and the statistician should all be maintained under blindness before unblinding. Through unblinding to the initiation of immune persistence observation in the recommended-dose group, the sponsors and statistician will no longer be blind while the subjects and investigators should remain blind. The testing facility will remain blind until all tests are completed.

6.10.3 Blinding of Vaccines

The randomization statistician and other personnel not involved in the clinical study will perform the vaccine blinding, that is, to post the printed vaccine labels in the designated location of each vaccine according to the blinding codes. The vaccine number will consist of the study number and the suffix of dose number. The randomization statistician should supervise the vaccine blinding and guide the blinding operators to label according to the blinding codes. At the end of blinding, the blinding codes shall be sealed by the randomization statistician in duplicate to be kept by the sponsor and the investigator, respectively. The whole process of blinding should be documented. The blinding personnel should not participate in other relevant work of this clinical trial, nor should they disclose the blinding codes to any personnel participating in this clinical trial.

6.10.4 Access to Backup Vaccines

The randomization statistician shall simultaneously prepare the backup vaccine randomization table and blinding the backup vaccines when generating the vaccine blinding codes. The vaccine randomization table should be uploaded into the backup vaccine acquisition system.

When the backup vaccine is needed for a subject whose study number is in the randomized blinding number range, the investigator will log in to the backup vaccine number acquisition system to obtain the corresponding vaccine number according to the operating procedure, and then the vaccine administrator will take the corresponding vaccine according to the operating procedure.

6.10.5 Emergency Letter and Emergency Unblinding

The randomization statistician will prepare 10 emergency letters during blinding. Each letter contains a randomized unblinding code, which corresponds to any vaccine number. The actual group corresponding to the vaccine number can be disclosed through the online unblinding system. Each code is for one operation of unblinding, and shall only be used for unblinding one vaccine number. The code will become invalid upon unblinding and the code is of no avail for any vaccine number which has already been unblinded. Emergency letters shall be provided and sent to the study site along with the blinded vaccines, and kept by the investigator. All emergency envelopes should be collected after the trial is completed, and the status of envelopes (opened or still sealed) will be checked at the blindness review meeting.

If a subject experiences a serious adverse event, and the information of experimental vaccines is essential for the clinical treatment or health of the subject, an individual emergency unblinding may be granted after confirmation by the Principal Investigator and the sponsor, that is, the emergency letter shall be opened for emergency unblinding according to the procedure and relevant records shall be made; then the serious adverse event shall be reported to the sponsor and Ethics Committee in a timely manner (within 24 hours). Subjects with corresponding study number will be terminated for the trial and determined as drop-out, and the investigator will record the reasons for termination in the Source Document. The emergency letters that have been opened should be properly kept and returned to the sponsor after the end of the trial. If emergency unblinding is required, the director of the study site shall open the emergency letter, log in to the online emergency unblinding system with the randomized unblinding code in the letter, and perform emergency unblinding as prompted.

In case of group adverse events or interruption of the trial for any reason, unblinding shall be performed in advance with the approval from the sponsor.

6.10.6 Provisions for Unblinding

After all subjects complete the 28-day visits post complete series, the safety data of subjects in the 5 μg dose group to 25 μg dose group after Dose 1 to 28 days after complete series will be locked after the blinded data are reviewed and the database is confirmed to be reliable and accurate. Then the unblinding will be performed and a record of unblinding will be kept. After unblinding, the testing facility and the study site shall remain blind for the follow-up visits.

6.10.7 Maintenance of Blindness

After unblinding of the safety data obtained from subjects in the 5 μg dose group to 25 μg dose group post Dose 1 to 28 days post complete series, the investigator for safety evaluation and laboratory test personnel will remain blind, and any information that may cause the leakage of blinding codes shall not be disclosed to them, so as to ensure the objectivity of the data.

6.11 Halting or Termination Rules of the Study

- **Halting rules for Sentinels**

During the safety observation period after Dose 1, if any of the following halting rules are triggered, a DSMB meeting will be held for safety assessment:

7) Vaccine-related death or life-threatening serious adverse events occur in any of the sentinels in the corresponding dose group.

8) Vaccine-related ulceration, abscess or necrosis at the injection site occur in any of the sentinels in the corresponding dose group.

9) Severe acute allergic reactions such as laryngospasm, tracheospasm, systemic edema combined with hypotension or gastrointestinal spasm occurs in any of the sentinels in the corresponding dose group within 24 hours after administration.

10) Systemic urticaria occurs in any of the sentinels in the corresponding dose group within 72 hours after administration (systemic reaction is defined as having 3 or more sites of the body observed with the symptom).

11) Any sentinel in the corresponding dose group experiences a fever with a body temperature > 40 ℃ within 7 days after administration.

12) More than 2 sentinels in the corresponding dose group experience an adverse event with a severity grade of 3 or above, lasting for > 48 hours without remission (remission: the adverse event is resolved after reasonable interventions* administered by investigators or its severity reduces to below grade 3 without any intervention), and is possibly/definitely related to vaccination or without reasonable attribution to causes other than vaccination.

If a dose group triggers the halting rule for sentinels, all subjects in that group and groups of higher doses should be halted for subsequent study procedures.

- **Halting rules for the study**

In case of any of the following situation occurs during the 28-day safety observation, a DSMB meeting shall be held to review whether to continue the study:

7) Vaccine-related death or life-threatening serious adverse events occur in any of the subjects in the corresponding dose group.

8) Ulceration, abscess, or necrosis at the injection site related to vaccination occur in any subject in the corresponding dose group.

9) Severe acute allergic reactions such as laryngospasm, tracheospasm, systemic edema combined with hypotension or gastrointestinal spasm occurs within 24 hours after vaccination in any subject in corresponding dose group.

10) Systemic urticaria occurs in ≥ 3 subjects in the corresponding dose group within 72 hours after vaccination (systemic reaction is defined as having 3 or more sites of the body observed with the symptom).

11) Any subject in the corresponding dose group experiences a fever with a body temperature > 40 ℃ within 7 days after vaccination.

12) After vaccination, the subject in the corresponding dose group experiences an adverse event with a severity grade of 3 or above, lasting for > 48 hours without remission (remission: the adverse event is resolved after reasonable interventions* administered by investigators or its severity reduces to below grade 3 without any intervention), and is possibly/definitely related to vaccination or without reasonable attribution to causes other than vaccination.

If a dose group triggers the halting rules for the study, all subjects in that group and groups of higher doses should be halted for subsequent study procedures.

*:"reasonable interventions" means the relevant measures specified in the Risk Control Plan for ADR.

6.12 Study Duration

Each subject will participate in the study for approximately 13 months.

7 Inclusion and Exclusion Criteria

7.1 Inclusion Criteria

Subjects are required to meet all of the following inclusion criteria:

1) Healthy adults aged 18-59 years and are able to provide legal identity certificate. Both male and female should be included.
2) The subjects shall fully understand the content of the informed consent form and the characteristics of the study vaccine, voluntarily sign the informed consent form, as well as have the ability to use thermometer, scale and fill in diary card and contact card as required.
3) The subjects shall be able to communicate well with the investigators, as well as understand and comply with the requirements of this study.
4) The subjects' axillary temperature on the day of administration should be less than 37.3℃.

7.2 Exclusion Criteria

7.2.1 Exclusion Criteria for the First Dose

Subjects who meet any of the following exclusion criteria should be excluded from the study:

1) Individuals whose results from a complete physical examination do not meet the healthy standards, mainly include:
   - Abnormal vital signs with clinical significance (pulse < 50/min or > 100/min, systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg).
   - BMI <18 kg/m^2 or >30 kg/m^2.
   - In the screening window, the laboratory test value exceeds 1.2 times of the upper limit or lower limit of the normal reference range, and is determined by the investigator to be clinically significant.
2) Women with positive results of pregnancy test (those who are in menstrual period or undergo amenorrhea for at least 1 year or is surgically sterilized with evidenced medical records can be exempted from pregnancy test), or women in lactation period, or women who plan to conceive from screening period to 12 months after complete series, men whose spouse plans to conceive, or those who plan to donate sperm and eggs.
3) Women in menstrual period (from the 1^{st} day to the 4^{th} day of menstrual cycle).
4) Individuals with history of vaccination with SARS-CoV-2 vaccines.
5) Individuals with prior diagnosis as COVID-19 cases or suspected cases. Individuals who have a history of contact with COVID-19 cases or suspected cases within 1 month before signing the informed consent, or those who have overseas travel history or have traveled to areas with high incidence of COVID-19; COVID-19 infector or carriers: serum SARS-CoV-2 specific antibody or SARS-CoV-2 nucleic acid is tested to be positive in throat swabs.
6) Those who are tested positive for hepatitis B surface antigen, hepatitis C core antigen, hepatitis C virus antibody, treponema pallidum specific antibody and human immunodeficiency virus antibody.
7) Those who have a history of SARS, MERS and other coronavirus infections or diseases in the past.

8) Those who have acute diseases or acute attack of chronic diseases within 2 weeks prior to the first dose of vaccination, or have fever (axillary temperature ≥ 37.3℃) or upper respiratory tract infection within 7 days prior to the first dose of vaccination.

9) Those who have a history of severe side effects induced by any vaccine or drug, such as allergy, urticaria, eczema, dyspnea, angioneurotic edema, etc.

10) Those who have been vaccinated with any vaccine within 1 month prior to Dose 1.

11) Those who cannot tolerate the venipuncture and have fainting history during acupuncture or at the sight of blood.

12) Those who have hereditary bleeding tendency or coagulation dysfunction, or have history of thrombosis or bleeding, and the detection results of coagulation function related measures are abnormal.

13) Those who have been diagnosed with congenital or acquired immunodeficiency (e.g., HIV infection).

14) Those who have asplenia or functional asplenia, or have other important organs removed by surgery for any reason.

15) Those who previously have serious diseases with abnormal clinical manifestations that needed to be excluded, including but not limited to a history of systemic diseases of nervous system, cardiovascular system, blood and lymphatic system, immune system, kidney, liver, gastrointestinal tract, respiratory system, metabolic and skeletal system, and malignant neoplasms (excluding controllable and stable chronic diseases such as diabetes, hypertension, etc.).

16) Those who have undergone surgery within 3 months prior to the signing of informed consent, or who plan to have surgery during the trial period or within 3 months after the end of the trial (including cosmetic surgery, dental surgery, and oral surgery).

17) Those who had blood donation or blood loss (≥450 mL), blood transfusion or use of blood products within 3 months prior to the signing of the informed consent, or plan to donate blood during the trial period.

18) Those who used any investigational or unlicensed products (drug, vaccine, biological product or device) other than the study vaccine within 3 months prior to the signing of the informed consent, or who plan to use the above-mentioned products during the study period.

19) Those who received immunosuppressive therapy within 6 months prior to signing the informed consent, such as long-term systemic glucocorticoid therapy (more than 2 weeks of continuous systemic glucocorticoid therapy, such as prednisone or similar drugs, within 6 months). Topical application (such as ointment, eye drops, inhalants, or nasal sprays) is allowed. Local medication should not exceed the dosage recommended in the prescribing information and the subjects should not have any signs of systemic exposure.

20) Those who are determined by the investigator to be not suitable to participate in the trial.

7.2.2 Exclusion Criteria for Dose 2

If any of the following conditions occur during the administration, vaccination for subjects should be postponed or terminated, but other study procedures can be continued according to the discretion of the investigator. Dose 2 should be administered 28 days after Dose 1 with a window of + 5 days:

1) Those who with positive results of urine pregnancy test.

2) For women in menstrual period (from the 1st day to the 4th day of menstrual cycle), Dose 2 should be postponed to the 5th day (inclusive) of the menstrual cycle.

3) Those who develop serious allergic reactions or serious adverse events with causal relationship with vaccination after Dose 1.

4) Those who are administered with any vaccine other than the study vaccine after Dose 1.

5) Those who have close contact with COVID-19 cases after Dose 1.

6) Those who are determined by the investigator to be not suitable to participate in the trial.

7.3 Criteria for Early Termination of Study

1) If the subject develops disability, life-threatening and other adverse events or serious adverse events, the investigator shall determine if the subject's participation in the study should be terminated according to the need for treatment.

2) The subjects voluntarily ask to withdraw from the clinical study, or their health status does not allow them to continue to participate in the study.

3) Other situations where the investigator determines the subjects not suitable to continue participating in the study.

7.4 Withdrawal of Subject

Subject withdrawal refers to that the subject cannot continue to complete the visits specified in the protocol due to meeting the criteria for early termination of the study or being unable to be contacted, including but not limited to:

1) The subject cannot continue to complete the subsequent visits due to meeting the criteria for early termination in 7.3.

2) The subject cannot be contacted at the visit time predetermined in the study protocol, and it is confirmed that the subject cannot attend all subsequent visits.

3) The subject permanently leaves the original place of residence and loses contact.

The investigator shall inform the subject of the right to withdraw from the study at any time. Withdrawn subjects will not be replaced, and the investigator shall make every effort to contact the subject who fails to return for follow-up as scheduled. All data collected before the withdrawal date may be used for analysis. The date of and possible reasons for withdrawal of the subject shall be recorded in the Original Record Book and eCRF, and the specific conditions shall be detailed:

1) Serious adverse events

2) Adverse event (but not serious)

3) Protocol violation

4) Voluntary withdrawal not due to adverse event

5) Going out, relocation or leaving from the study site

6) Lost to follow-up

7) Death

8) Others

Subjects who withdraw from the study due to adverse events must be clearly distinguished from those who withdraw from the study due to other reasons. After withdrawal or termination of a subject who receives the investigational vaccine or placebo control, the investigator shall provide necessary treatment for the subject with clinical conditions related to the study, and follow up adverse events/serious adverse events until definite diagnosis/stable disease/recovery/return to baseline.

Time for subject withdrawal: The time when the investigator decides to terminate the study for a subject or when the subject voluntarily withdraws from the study.

Definition of subject's completion of clinical study: A subject is considered to have completed the clinical study when he/she completes the 12-month safety observation after complete series and blood collection for immunogenicity specified in the protocol.

8 Lost to Follow-up, Protocol Deviation/Violation and Pregnancy-related Events in Subjects

8.1 Handling of Subjects Lost to Follow-up

When a subject is unable to return on time for a follow-up visit, with full respect for the rights of the subject, the investigator shall make efforts to contact the subject for confirmation, recall the subject, or at least determine the subject's health status, and record the measures of efforts made (e.g., telephone calls and SMS records).

8.2 Protocol Deviation/Protocol Violation

Protocol deviation: It refers to any change or non-compliance to the design or procedure of the clinical trial protocol without approval from the Ethics Committee. The behaviors that do not affect the rights, safety and benefits of the subjects, or the integrity, accuracy, and reliability of the trial data as well as the evaluation on the safety or main indicators are protocol deviations. The behaviors that affect the rights, safety and benefits of the subjects, or the integrity, accuracy, and reliability of the trial data as well as the evaluation on the safety or main indicators are serious protocol deviations (protocol violations).

Deviations from this study protocol include, but are not limited to:

- Vaccination or blood collection is performed out of the window period
- The volume of blood collected for humoral immunity or cellular immunity tests exceeds the prescribed upper limit

Violations of this study protocol include, but are not limited to:

- Failure to provide fully informed consent to subjects.
- Subjects who do not meet the inclusion criteria or meet the exclusion criteria are enrolled into the study
- Failure of the investigator to timely terminate the study for subjects who have met the conditions for study termination
- Subjects receive wrong intervention (such as vaccination error)
- Subjects fail to complete blood collection
- The blood collection volume is insufficient and affects the humoral immunity tests or the serum in any tube submitted for testing is less than 0.5 mL
- The volume of blood collected is insufficient and affects the evaluation for the exploratory endpoints of specific cellular immunity or the volume of heparin sodium-anticoagulated blood samples in any tube submitted for testing is less than 4.0 mL
- SAE is not reported or not reported in time
- Vaccination with other vaccines before the completion of two doses of the investigational vaccine or < 28 days after the completion of two doses of the investigational vaccine
- Pregnancy and related events occur from Dose 1 to 12 months after complete series

For protocol violations/deviations, the investigator should report to the Principal Investigator and sponsor in a timely manner, render special attention to subjects involved, collect safety information, ensure the safety of subjects, and record the event in detail.

8.3 Pregnancy-related Events

The investigator should collect the pregnancy-related events from Dose 1 to 12 months after complete series, fill in the Pregnancy-related Event Report Form after learning of the pregnancy of the subject/subject's spouse, and report the to (1) PI (2) sponsors (3) CRA by telephone, fax, or email within 5 natural days. All pregnant women during the collection period of pregnancy-related events should be followed up until the end of pregnancy, and the outcomes should be recorded, including pregnancy outcomes, delivery characteristics (pregnancy duration, outcome, delivery), condition of the neonates (gender, weight, height, neonatal score), as well as growth and development within 12 months after birth. In this trial, pregnancy itself is not considered as a serious adverse event, while any complication during pregnancy is considered as an adverse event, and may be considered as a serious adverse event in some cases, such as spontaneous abortion, dead fetus, stillbirth, and congenital anomaly of the infant.

9 Vaccines, Transportation & Storage of Vaccines and Guidance for Vaccination

9.1 Vaccine Information

Investigational vaccine:	SARS-CoV-2 mRNA vaccine (ARCoV)
Manufacturer:	Suzhou Abogen Biosciences Co., Ltd.
Specification and composition:	5 μg, 0.5mL/vial, containing 5 μg mRNA
	10 μg, 0.5mL/vial, containing 10 μg mRNA
	15 μg, 0.5mL/vial, containing 15 μg mRNA
	20 μg, 0.5mL/vial, containing 20 μg mRNA
	25 μg, 0.5mL/vial, containing 25 μg mRNA
Description:	clear colorless liquid
Batch No. and expiration date:	5 μg, AE2008001, Aug 11, 2022
	10 μg, AD2008001, Aug 10, 2022
	15 μg, AC2008001, Aug 09, 2022
	20 μg, AB2008001, Aug 12, 2022
	25 μg, AA2008001, Aug 08, 2022
Placebo:	Normal saline (0.9% sodium chloride solution)
Development institution:	Suzhou Abogen Biosciences Co., Ltd.
Specification and composition:	0.5 mL/vial, 0.9% sodium chloride solution;
Description:	clear colorless liquid
Batch No. and expiration date:	D2005001, February 2022.

9.2 Storage and Transportation of Vaccines

Vaccines should be stored and transported at 2℃ to 8℃ with protection from light. The vaccines should be strictly on guard against freezing. The storage and transportation temperature should be monitored and recorded according to the SOPs for vaccine management or relevant system. If the temperature is > 8℃ during the vaccine storage or transportation and the duration does not exceed 30 minutes, it is considered as "cold chain violations"; if the temperature is < 2℃ during the vaccine storage or transportation (regardless of the duration), or the temperature is > 8℃ during the vaccine storage or transportation and the duration exceeds 30 min, it is considered as "cold chain failure". If any of the above conditions occur during the storage or transportation of the vaccines, the investigator should immediately move the vaccine to a dark environment at 2℃ -8℃ with protection from light, immediately contact the CRO and the sponsor, and issue a cold chain violation or cold chain failure report. The involved vaccines should not be used before the written comments are obtained from the sponsor.

9.3 Site, Route, Dose and Schedule for Immunization

Administration site and route: Intramuscular injection into the lateral deltoid of the upper arm.

Doses: 5 μg dose, 0.5 mL/dose

10 μg dose, 0.5 mL/dose

15 μg dose, 0.5 mL/dose

20 μg dose, 0.5 mL/dose

25 μg dose, 0.5 mL/dose

Immunization schedule: 1 dose on D0 and D28 respectively, 2 doses in total.

9.4 Backup Vaccines

In this clinical trial, in addition to the investigational vaccines required for normal vaccination, 25% of the actual amount of vaccines required will be prepared as backup vaccines for the investigational vaccine at each dose and control placebo, i.e., 10 vials for the investigational vaccine at doses of 5 μg, 10 μg, 15 μg, 20 μg and 25 μg, respectively, and a total of 10 vials for the placebo (normal saline). In case of any damage, precipitation, insufficient load, abnormal appearance, etc., observed for the experimental vaccine during the study, the investigator can log in to the backup vaccine acquisition system to obtain the backup vaccine number.

9.5 Vaccine Number

The "vaccine number" for this clinical trial consists of the study number and a suffix of dose number. For example, the vaccine number of Dose for subject with the study number of 001 is 001-1. The backup vaccine number consists of letter "B" and the suffix number, which contains randomization information, and the number segment will be B01-B60.

9.6 Vaccine Assignment

Study numbers will be assigned according to the sequence in which the screening eligible subjects are enrolled. The screening number and the subject’s initials will be entered into the *Randomization Table*, and experimental vaccines will be obtained corresponding to the study number.

9.7 Vaccine Packaging

To maintain standardized management and proper use of vaccines, the experimental vaccines in this clinical trial will be numbered and uniformly packaged in white small and medium boxes. The strength of the repackaged vaccine is 1 vial/small box, 20 vials/medium box. Medium boxes with less than 20 vials will be fully filled with empty small boxes after experimental vaccines and backup vaccines are filled in in sequence. Vaccines will be marked with vial labels containing a unique number and small box labels, stickers with the corresponding number will be placed in the small box, and labels with corresponding number segment will be used to identify the medium box.

9.8 Vaccine Labels

In addition to the vial labels and small box labels with the same number which are pasted on the pre-filled syringe and the small box, respective, a pre-printed sticker with the same number will be put in the small box to be pasted on the corresponding field for vaccination information in the subject’s *Source Document*. Labels for Dose 1, Dose 2 and backup vaccines are distinguished by white, light yellow and green.

9.8.1 Vial Label

Vial labels for experimental vaccines (including the investigational vaccine and the placebo) are as follows:

SARS-CoV-2 mRNA Vaccine
Vaccine No.: 001-1, Dose 1
(For Clinical Study Only)

SARS-CoV-2 mRNA Vaccine
Vaccine No.: 001-2, Dose 2
(For Clinical Study Only)

SARS-CoV-2 mRNA vaccine
Vaccine No.: B01
(For Clinical Study Only)

9.8.2 Small Box Label

Small box label for experimental vaccines (including the investigational vaccine and the placebo) are as follows:

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Vaccine No.: 001-1 Dose No.: Dose 1
Initials: Vaccination date:
For clinical study only
Store and transport at 2 ℃ -8 ℃, protect from light
Academy of Military Medical Sciences
Suzhou Abogen Biosciences Co., Ltd., Walvax Biotechnology Co., Ltd.

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Vaccine No.: 001-2 Dose No.: Dose 2
Initials: Vaccination date:
For clinical study only
Store and transport at 2 ℃ -8 ℃, protect from light
Academy of Military Medical Sciences
Suzhou Abogen Biosciences Co., Ltd., Walvax Biotechnology Co., Ltd.

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Vaccine No.: B01
Initials: Vaccination date:
For clinical study only
Store and transport at 2 ℃ -8 ℃, protect from light
Academy of Military Medical Sciences
Suzhou Abogen Biosciences Co., Ltd., Walvax Biotechnology Co., Ltd.

9.8.3 Stickers in the Small Box

Stickers in the small box for experimental vaccines (including the investigational vaccine and the placebo) are as follows:

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Vaccine No.: 001-1, Dose 1
Valid until
(For Clinical Study Only)

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Vaccine No.: 001-2, Dose 2
Valid until
(For Clinical Study Only)

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Vaccine No.: B01
Valid until
(For Clinical Study Only)

9.8.4 Medium Box Labels

Medium box labels for experimental vaccines (including the investigational vaccine and the placebo) are as follows:

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Dose No.: Dose 1 Numbers of vaccines in the box (001-1~020-1)
Batch No.:
Valid until: (MM/YYYY)
For clinical study only Store and transport at 2 ℃-8 ℃, protect from light
Academy of Military Medical Sciences
Suzhou Abogen Biosciences Co., Ltd., Walvax Biotechnology Co., Ltd.

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Dose No.: Dose 2 Numbers of vaccines in the box (001-1~020-1)
Batch No.:
Valid until: (MM/YYYY)
For clinical study only Store and transport at 2 ℃-8 ℃, protect from light
Academy of Military Medical Sciences
Suzhou Abogen Biosciences Co., Ltd., Walvax Biotechnology Co., Ltd.

SARS-CoV-2 mRNA Vaccine (ARCoV-002)
Numbers of vaccines in the box (B01-B20)
Batch No.:
Valid until: (MM/YYYY)
For clinical study only Store and transport at 2 ℃-8 ℃, protect from light
Academy of Military Medical Sciences
Suzhou Abogen Biosciences Co., Ltd., Walvax Biotechnology Co., Ltd.

10 Concomitant Medications

Concomitant medications: They refer to all the drugs except for experimental vaccines that subjects receive within 0-28 days after vaccination, including antibiotics, antiviral drugs, antipyretic and analgesic drugs, anti-allergic drugs, biological products (vaccines, immunoglobulins, blood products, etc.), Chinese (patent) drugs (excluding non-therapeutic vitamins and/or food additives) and glucocorticoids. The information of concomitant medications, including drug name, usage, and dosage, starting and ending time of use, etc., should be recorded.

Permitted medications: During the study, subjects should be allowed to receive necessary drug therapy in case of any adverse event.

Permitted vaccines: Vaccines other than emergency vaccines (such as tetanus vaccine or rabies vaccine) shall not be administered from the first dose to 28 days post complete series. If any other vaccines are administered before completing 2 doses of experimental vaccines, a "protocol violation" will be recorded and reported. After completing 2 doses of experimental vaccines, in case of emergency vaccination, concomitant vaccines can be administered according to the instructions prescribed in the product package insert, with close monitoring for safety and detailed records made. A "protocol violation" should be recorded and reported if the interval between emergency vaccination and the completion of experimental vaccines is< 28 days.

Medication records: To understand the effect of the drugs used during the study on the safety of the vaccine, or to collect the adverse events that may be related to vaccination without omission, the investigator should take measures to collect any medication used from the subjects during the observation period, instruct the subjects to record all medical attention and medications received within 0-28 days after vaccination in the *Diary Card* and *Contact Card* if possible, and review the medications recorded by the subjects.

11 Study Methods and Procedures

11.1 Recruitment of Volunteers

A recruitment notice will be distributed to populations that meet the requirements on age, vaccination history, and health status prescribed in the protocol after the study site is determined and approval from the Ethics Committee is obtained.

11.2 Study Process

Visits 0-11 are required for all subjects included in this trial, and visits 12-15 are required for subjects included in the study group of the recommended dose. Additional blood collection is required for subjects in the specific cellular immunogenicity subgroup during visits 1, 4, 9, 10, and 11, as well as for those in the study group of the recommended dose who are included for specific cellular immunogenicity evaluation during visits 12 and 13.

11.2.1 Visit 0 (D -7 to D 0)

Sign the informed consent form and complete screening tests.

1) Registration

The investigator responsible for registration shall verify volunteers' identity information and age, distribute them a serial number (consisting of capital letters "LS" and three sequential digits, such as LS001), and issue the *Circulation Form*.

2) Informed consent and assignment of screening numbers

The investigators shall fully inform volunteers of the relevant information of the clinical study. Volunteers agreeing to grant informed consent should sign the *Informed Consent Form* with the investigator initiating the informed consent. The *Informed Consent Form* is in duplicate, with the original kept by the study site and the duplicate kept by the volunteers. Volunteers not agreeing to grant informed consent do not need to sign the *Informed Consent Form*, and the study center shall not keep any information of them. In addition, the investigators should sign their names in the Informed Consent position on the *Circulation Form* and specify details in the blank space.

After signing the *Informed Consent Form*, the investigators will assign a screening number (consisting of capital letter S and three digits, for example, the screening number of the first volunteer is "S001") to the volunteers and copy the volunteers' identification documents.

3) Detection of SARS-CoV-2 nucleic acid and specific antibodies

Throat swabs of volunteers granting informed consent will be collected for SARS-CoV-2 nucleic acid detection, and blood samples will be collected for the detection of specific antibodies against SARS-CoV-2. If the above two detection results of volunteers are both negative, the investigators shall fill in the corresponding section of the *Source Document* with relevant results.

4) Physical examination and consultation screening

The investigators will perform a routine physical examination on volunteers granting informed consent; observe their general conditions, including axillary temperature, height, body weight, pulse, and blood pressure. The blood samples and urine samples will be collected for blood biochemistry, hematology, coagulation, urinalysis laboratory tests, blood pregnancy test (for women of childbearing age only) and blood-borne infectious diseases tests (including hepatitis B, hepatitis C, syphilis, AIDS-related etiology, or serological screening). See Annex 3 for specific detectable items. The volunteers will be inquired based on the "inclusion and exclusion criteria" and their history of allergy, disease, and medication, etc. will be collected. The investigators shall fill in the corresponding section of the *Source Document* with the volunteers' physical examination

and consultation results and determine the eligibility of volunteers to be included in this clinical study.

5) Remind eligible volunteers to attend the next visit.

11.2.2 Visit 1 (D0)

1) Physical examination and consultation screening

The volunteer's axillary temperature will be measured. Urine samples will be collected from women at childbearing age for pregnancy test (those who had amenorrhea for at least 1 year or had medical records of surgical sterilization are exempted from pregnancy test). The investigators shall further screen the volunteers according to the "inclusion and exclusion criteria", fill in the corresponding section of the *Source Document* with the volunteers' body temperature and inquiry information, and determine the eligibility of volunteers to be enrolled in the clinical study.

2) Assignment of study numbers

After physical examination and consultation, volunteers who are determined by investigators to be eligible for this clinical study are included. The investigators will fill the initials and screening numbers of the enrolled subjects in the *Randomization Form*, as well as sign names and the date; assign the subjects a study number according to the order of inclusion, and fill the study number in the *Source Document.*

In consideration of the practical operability and scientific design of the trial, volunteers are allowed to have SARS-CoV-2 nucleic acid detection, SARS-CoV-2 antibody detection, blood biochemistry, hematology, coagulation, blood pregnancy test (for women at childbearing age only), and blood-borne infectious disease screening on the day of Dose 1 (D0). However, the study numbers can only be assigned after all test reports are analyzed by investigators to meet the inclusion and exclusion criteria of this study.

3) Collection of pre-immunization blood samples for immunogenicity evaluation

Venous blood will be collected from subjects for immunogenicity tests:

a Humoral immunity tests: about 6.0 mL of blood will be collected with a pro-coagulation tube (containing coagulant only), the serum will be separated according to the operating procedures and placed in a cryovial, in which 0.5 mL of the serum will be removed to Tube A for the detection of neutralizing antibody against SARS-CoV-2 (pseudovirus neutralization test), 0.5 mL of the serum to Tube B for the detection of neutralizing antibody against SARS-CoV-2 (wild-type virus neutralization test), 0.5 mL of the serum to Tube C for specific IgG antibody detection, and the remaining serum will be kept as backup and stored in Tube D.

Requirements for the blood volume collected for humoral immunity tests: If the blood volume collected via a tube for humoral immunity is > 7.0 mL, a protocol deviation will be reported; if the separated serum volume does not reach 1.5 mL (serum sent for testing in any tube is less than 0.5 mL), a protocol violation will be reported.

b Detection of exploratory endpoints for specific cellular immunity (only applicable to subjects included in the specific cellular immunogenicity subgroup): five heparin sodium anticoagulation tubes (Tubes E, F, G, H, and I) will be used to collect venous blood respectively (about 4.0 mL/tube, 20.0 mL in total), and then stand at 2 ℃-8 ℃. Tubes E - H will be used to test the proportion of specific immune cell subsets and cytokine levels, while Tube I will be used to test the proportion of memory T cells.

Requirements for the blood volume collected for specific cellular immunity tests: If the blood volume of any tube collected from subjects included in the specific cellular immunogenicity subgroup is > 5.0 mL, a protocol deviation will be reported; if the blood volume of any tube is less than 4.0 mL, a protocol violation will be reported.

4) Vaccination

The vaccine preparation personnel shall obtain the corresponding vaccine number in accordance with the study number of the subject, verify that the information on the small box label and the small box stickers of the vaccine is correct and consistent, and confirm that the vaccine is in normal status. After verifying the study number on the *Source Document* with the subjects, the vaccinator shall fill in the initials of the subjects and the date of vaccination on the label of the small box; take a small box sticker and paste it on the designated position on the *Source Document*, and fill in the initials of the subjects.

The vaccinator shall verify the subject information again before vaccination for each subject; after verifying, disinfect the skin of the lateral deltoid muscle of the subject's upper arm with 75% medical alcohol, fully shake the vaccine well, and intramuscularly inject the vaccine after the skin is slightly dry. After vaccination, the investigator shall fill in the vaccination time, vaccination site and other information to the corresponding position on the *Source Document*, and sign the name and the date.

5) Safety observation after immunization

All subjects will be medically observed at the study site for 60 min after vaccination, and adverse events will be recorded. The investigator shall provide the subject with *Diary Card* 1, a thermometer and a scale, train the subject how to grade the adverse events by using the thermometer and scale as well as how to fill in *Diary Card* 1, and make an appointment to return *Diary Card* 1 with the subject.

The investigators shall highlight the investigator's phone number on the copy of the *Informed Consent Form* and on *Diary Card* 1 to the subjects, and instruct them to contact the investigators immediately if they experience any signs, symptoms, or events requiring hospitalization that they considered serious after vaccination.

11.2.3 Visit 2 (the 1st day pots Dose 1 with a time window of + 1 day)

1) Hematology, blood biochemistry and coagulation laboratory tests

Venous blood will be collected from subjects for blood biochemistry, hematology, and coagulation tests.

2) Urine collection for laboratory tests

Urine samples will be collected from subjects for urinalysis.

11.2.4 Visit 3 (the 4th day post Dose 1 with a time window of + 1 day)

1) Hematology, blood biochemistry and coagulation laboratory tests

Venous blood will be collected from subjects for blood biochemistry, hematology, and coagulation tests.

2) Urine collection for laboratory tests

Urine samples will be collected from subjects for urinalysis.

11.2.5 Visit 4 (the 7th day post Dose 1 with a time window of + 1 day)

1) Hematology, blood biochemistry and coagulation laboratory tests

Venous blood will be collected from subjects for blood biochemistry, hematology, and coagulation tests.

2) Urine collection for laboratory tests

Urine samples will be collected from subjects for urinalysis.

3) Blood collection for the detection of exploratory endpoints for specific cellular immunity (only applicable to subjects included in the specific cellular immunogenicity subgroup)

Four heparin sodium anticoagulation tubes (Tubes E, F, G and H) will be used to collect about 4.0 mL/ tube of venous blood respectively (16.0 mL in total), and then stand at 2℃ -8℃, all of which will be used to test the proportion of specific immune cell subsets and cytokine levels. The requirement for blood volumes is the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation - detection of exploratory endpoints for specific cellular immunity ".

11.2.6 Visit 5 (the 15th day post Dose 1)

1) Collection and review of *Diary Card* 1

The subjects will return *Diary Card* 1. The investigators shall review and confirm with the subjects whether the descriptions of adverse events or concomitant medications are completely and correctly recorded in *Diary Card* 1, and instruct the subjects to correct and supplement the contents filled in if necessary before collecting Diary Card 1.

2) Training and distribution of *Contact Card* 1

The investigators shall distribute *Contact Card* 1 to the subjects; train them to record all adverse events and concomitant medications (if any) during 15-28 days after Dose 1 in *Contact Card* 1.

11.2.7 Visit 6 (the 28th day post Dose 1 with a time window of + 5 days)

1) Collection and review of *Contact Card* 1

The subjects will return *Contact Card* 1. The investigators shall review and confirm with the subjects whether the descriptions of adverse events or concomitant medication are completely and correctly recorded in the *Contact Card* 1, and instruct the subjects to correct and supplement the contents filled in if necessary before collecting *Contact Card* 1.

2) Physical examination and screening for Dose 2

The subject's axillary temperature will be measured. Urine samples will be collected from women at childbearing age for pregnancy test (those who had amenorrhea for at least 1 year or had medical records of surgical sterilization are exempted from pregnancy test). The investigators shall screen the subjects in accordance with the "exclusion criteria for the second dose", fill in the physical examination and screening results of the subjects in the corresponding part of the *Source Document*, and determine whether the subjects can continue with the Dose 2.

3) Collection of post-immunization blood samples for humoral immunity tests

The specific operation and requirements are the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation-humoral immunity tests".

4) Blood collection for laboratory tests

Venous blood will be collected from subjects for blood biochemistry, hematology, and coagulation tests.

5) Urine collection for laboratory tests.

Urine samples will be collected from subjects for urinalysis.

6) Vaccination

Dose 2 of the investigational vaccine will be vaccinated in accordance with the procedures of "Vaccination" in Visit 1.

7) Safety observation after immunization

Safety observation will be performed in accordance with the procedures of "Safety Observation after Immunization" in Visit 1.

8) Training and distribution of *Diary Card* 2

The investigators shall distribute *Diary Card* 2 to the subjects and make an appointment to return *Diary Card* 2 with the subjects.

11.2.8 Visit 7 (the 1st post Dose 2 with a time window of + 1 day)

1) Blood collection for laboratory tests

Venous blood will be collected from subjects for blood biochemistry, hematology, and coagulation tests.

2) Urine collection for laboratory tests.

Urine sample will be collected from subjects for urinalysis.

11.2.9 Visit 8 (the 4th day post Dose 2 with a time window of + 1 day)

1) Blood collection for laboratory tests

Venous blood will be collected from subjects for blood biochemistry, hematology, and coagulation tests.

2) Urine collection for laboratory tests.

Urine samples will be collected from subjects for urinalysis.

11.2.10 Visit 9 (the 7th day post Dose 2 with a time window is + 2 days)

1) Blood collection for laboratory tests

Venous blood will be collected from subjects for blood biochemistry, hematology, and coagulation tests.

2) Urine collection for laboratory tests

Urine samples will be collected from subjects for urinalysis.

3) Collection of post-immunization blood samples for humoral immunity tests

The specific operation and requirements are the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation-humoral immunity tests".

4) Blood collection for the detection of exploratory endpoints for specific cellular immunity (only applicable to subjects included in the specific cellular immunogenicity subgroup)

Four heparin sodium anticoagulation tubes (Tubes E, F, G and H) will be used to collect about 4.0 mL/ tube of venous blood respectively (16.0 mL in total), and then stand at 2 ℃ -8 ℃, all of which will be used to test the proportion of specific immune cell subsets and cytokine levels. The requirement for blood volumes is the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation - detection of exploratory endpoints for specific cellular immunity ".

11.2.11 Visit 10 (the 15th day post Dose 2 with a time window of + 2 days)

1) Collection and review of *Diary Card* 2

The subjects will return *Diary Card* 2. The investigators shall review with the subjects whether the descriptions of adverse events or concomitant medications are completely and correctly recorded in *Diary Card* 2, and instruct the subjects to correct and supplement the contents filled in if necessary before collecting the *Diary Card* 2.

2) Collection of post-immunization blood samples for humoral immunity tests

The specific operation and requirements are the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation-humoral immunity tests".

Blood collection for the detection of exploratory endpoints for specific cellular immunity (only applicable to subjects included in the specific cellular immunogenicity subgroup)

Four heparin sodium anticoagulation tubes (Tubes E, F, G and H) will be used to collect about 4.0 mL/ tube of venous blood respectively (16.0 mL in total), and then stand at 2 ℃ -8 ℃, all of which will be used to test the proportion of specific immune cell subsets and cytokine levels. The requirement for blood volume is the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation - detection of exploratory endpoints for specific cellular immunity ".

3) Training and distribution of *Contact Card* 2

The investigators shall distribute *Contact Card* 2 to the subjects; train them to record all adverse events and concomitant medications (if any) during 15-28 days after Dose 1 in *Contact Card* 2.

11.2.12 Visit 11 (the 28th day post complete series with a time window of + 2 days)

1) Collection and review of *Contact Card* 2

The subjects will return *Contact Card* 2. The investigators shall review with the subjects whether the descriptions of adverse events or concomitant medication are completely and correctly recorded in *Contact Card* 2, and instruct the subjects to correct and supplement the contents filled in if necessary before collecting *Contact Card* 2.

2) Blood collection for laboratory tests

Venous blood will be collected from subjects for blood biochemistry, hematology, and coagulation tests.

3) Urine collection for laboratory tests

Urine samples will be collected from subjects for urinalysis.

4) Collection of post-immunization blood samples for humoral immunity tests

The specific operation and requirements are the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation-humoral immunity tests".

5) Blood collection for the detection of exploratory endpoints for specific cellular immunity (only applicable to subjects included in the specific cellular immunogenicity subgroup)

A heparin sodium anticoagulation tube (Tube I) will be used to collect about 4.0 mL/ tube of venous blood (4.0 mL in total), and then stand at 2 ℃-8 ℃, which will be used to test the proportion of memory T cells. The requirement for blood volumes is the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation- detection of exploratory endpoints for specific cellular immunity ".

11.2.13 Immune Persistence Visit (Subjects in the Study Group of Recommended Dose)

- **Visit 12 (90 days post complete series with a time window of + 7 days)**

Collection of blood sample - 1 for immune persistence evaluation: The venous blood will be collected from the subjects for humoral immunity tests. The specific operation and requirements are the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation-humoral immunity tests".

Blood collection for the detection of exploratory endpoints for specific cellular immunity (only applicable to subjects included in the specific cellular immunogenicity subgroup): a heparin sodium anticoagulation tube (Tube I) will be used to collect about 4.0 mL/ tube of venous blood (4.0 mL in total), and then stand at 2 ℃ -8 ℃, which will be used to test the proportion of memory T cells. The requirement for blood volumes is the same as "collection of pre-immunization blood samples for immunogenicity evaluation- detection of exploratory endpoints for specific cellular immunity ".

- **Visit 13 (180 days post complete series with a time window of + 14 days)**

Collection of blood sample - 2 for immune persistence evaluation: The venous blood will be collected from the subjects for humoral immunity tests. The specific operation and requirements are the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation-humoral immunity tests".

Blood collection for the detection of exploratory endpoints for specific cellular immunity (only applicable to subjects included in the specific cellular immunogenicity subgroup): five heparin sodium anticoagulation tubes (Tubes E, F, G, H and I) will be used to collect about 4.0 mL/ tube of venous blood respectively (20.0 mL in total), and then stand at 2 ℃ -8 ℃, of which, Tubes E, F, G and H will be used to test the proportion of specific immune cell subsets and cytokine levels, and Tube I will be used to test the proportion of memory T cells. The requirement for blood volumes is the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation- detection of exploratory endpoints for specific cellular immunity".

- **Visit 14 (270 days post complete series with a time window of + 21 days)**

Collection of blood sample - 3 for immune persistence evaluation: The venous blood will be collected from the subjects for humoral immunity tests. The specific operation and requirements are the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation-humoral immunity tests".

- **Visit 15 (360 days post complete series with a time window of + 30 days)**

Collection of blood sample - 4 for immune persistence evaluation: The venous blood will be collected from the subjects for humoral immunity tests. The specific operation and requirements

are the same as stated in "collection of pre-immunization blood samples for immunogenicity evaluation-humoral immunity tests".

11.2.14 End of Study

After the EDC data entry is reviewed, the data is clarified, the database is locked, and all the data are reviewed, the Study Site Closure Report will be issued.

See Table 1 for the schedule and procedures of the study:

Table 1 Schedule for Subjects in Phase Ib Clinical Trial

On-site Visit #		From Dose 1 to 28 Days post Complete Series (Including the Exploratory Observation for Cellular Immunity[&])												Persistence * (Including Exploratory Endpoints for Cellular Immunity[$])			
		V0	V1	V2[##]	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
		D-7 to D0	D0	D1	D4[a]	D7	D15[b]	D28	D29	D32	D35	D43[c]	D56	V6 +90	V6 +180	V6 +270	V6 +360
Time Window		/	/	+1	+1	+1	/	+5	+1	+1	+2	+2	+2	+7	+14	+21	+30
1	Screening, informed consent, signing of Informed Consent Form, assignment of screening number	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
2	Collection of demographic data	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3	Screening for preliminary inclusion and exclusion criteria	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4	Physical examination:	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Height and weight	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Pulse and blood pressure	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Serum antibody test for SARS-CoV-2, Nucleic acid test of throat swab for SARS-CoV-2	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Test for blood-borne diseases [d]	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Blood pregnancy test (females of childbearing age only)	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Hematology, blood biochemistry,	X	/	X	X	X	/	X	X	X	X	/	X	/	/	/	/

	urinalysis, and coagulation																
	Urine pregnancy test (females of childbearing age only)	/	X	/	/	/	/	X	/	/	/	/	/	/	/	/	/
5	Axillary temperature measurement	X	X	/	/	/	/	X	/	/	/	/	/	/	/	/	/
6	Screening for inclusion and exclusion criteria	/	X	/	/	/	/	X	/	/	/	/	/	/	/	/	/
7	Enrollment and assignment of study number	/	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/
8	Immunogenicity: humoral immunity [e]	/	X	/	/	/	/	X	/	/	X	X	X	X	X	X	X
	Specific cellular immunity [&]	/	X[&]	/	/	X[&]	/	/	/	/	X[&]	X[&]	X[&]	X[$]	X[$]	/	/
9	Vaccination	/	X	/	/	/	/	X	/	/	/	/	/	/	/	/	/
10	On-site safety observation for 60 min. Train the subjects for safety observation and records.	/	X	/	/	/	/	X	/	/	/	/	/	/	/	/	/
11	Distribution of Diary Cards to the subjects	/	X	/	/	/	/	X	/	/	/	/	/	/	/	/	/
12	Distribution of scales and thermometers to the subjects	/	X	/	/	/	/	/	/	/	/	/	/	/	/	/	/
13	Review and collection of Diary Cards	/	/	/	/	/	X	/	/	/	/	X	/	/	/	/	/
14	Distribution of Contact Cards	/	/	/	/	/	X	/	/	/	/	X	/	/	/	/	/
15	Review and collection of Contact Cards	/	/	/	/	/	/	X	/	/	/	/	X	/	/	/	/
16	Adverse events	/	X	X	X	X	X	X	X	X	X	X	X	/	/	/	/

17	Concomitant medication	/	X	X	X	X	X	X	X	X	X	X	X	/	/	/	/
18	SAEs, SARS-CoV-2 infection, autoimmunity diseases or tumorigenesis	/	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
19	Pregnancy-related events [f]	/	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Note: V0 (D-7 to D0) and V1 (D0) can be performed on the same day.

#: In addition to the above on-site visits, all subjects will be required for 14 phone visits: once at 4~6 hours after each vaccination, once on the 2nd day after vaccination, once on the 3rd day after each vaccination and once on the 5th day after each vaccination, respectively (8 times in total); once for every 2 months after complete series (6 times in total).

##: "sentinel" subjects will be hospitalized for observation for 24 hours (1 day) after the Dose 1, and relevant tests for Visit 2 can be completed during the hospitalization.

*: On-site visits for immune persistence evaluation are only for subjects in the study group of the recommended dose.

&: Visits for cellular immunity exploratory endpoints are only for subjects included in the specific cellular immunogenicity subgroup. Blood samples will be collected before Dose 1 for the tests of the proportion of specific immune cell subsets, cytokine levels and the proportion of memory T cells. The tests of the proportion of specific immune cell subsets and cytokine levels will be performed 7 days after Dose 1, 7 days after complete series, and 15 days after complete series. The proportion of memory T cells will be tested on 28 days after complete series.

For subjects in the specific cellular immunogenicity subgroup participating in the visits for immune persistence evaluation, blood samples will be collected only 3 months after complete series for the test of the proportion of memory T cells, and blood samples will be collected 6 months after complete series for the test of the proportion of specific immune cell subsets, cytokine levels and the proportion of memory T cells.

a: The safety condition of "sentinels" in each dose group on D0 ~ D4 after Dose 1 is the basis for judging whether subsequent subjects in the same dose group and "sentinels" in the next dose group should be enrolled.

b: Safety data observed with 0-14 days post Dose 1 will be collected on the 15th day post Dose 1.

c: Safety data observed with 0-14 days post Dose 2 will be collected on the 15th day post complete series.

d: Tests for blood-borne diseases include qualitative test of hepatitis B surface antigen, hepatitis C virus core antigen, antibody to hepatitis C virus, syphilis-specific antibody, and HIV antibody assay.

e: The volume of blood samples for humoral immunity is about 6.0 mL/time.

g: The volume of blood samples for the tests of the proportion of specific immune cell subsets and cytokine levels is about 16.0 mL/time, and the volume of blood samples for the test of the proportion of memory T cells is about 4.0 mL/time.

f: Pregnancy-related events include pregnancy outcomes, delivery characteristics, delivery of newborns, as well as growth and development within 12 months after birth.

11.3 Safety Observation Method

11.3.1 Safety Observation Time and Method

(1) Within 60 minutes after each dose of the vaccine

After each dose, all subjects should undergo a 60-minute systematic medical observation on site by specially trained and authorized investigators, and observed adverse events should be recorded in the *Diary Card*.

(2) From Dose 1 to 28 days after complete series

After each dose, 60-minute medical observation should be carried out at the study site. A trained and authorized investigator will systematically observe each subject, measure his/her body temperature at 60 minutes after immunization, and record in detail all adverse events within 60 minutes after vaccination. On Days 0-14 after each dose, all adverse events observed will be systematically observed and recorded in the *Diary Card*; On Days 15-28 after each dose, adverse events found will be collected and recorded in the *Contact Card*.

After collecting the *Diary Card* and *Contact Card* for each dose, the investigator should verify their original safety information in time before entering relevant items into the EDC system. The investigator should verify the name, duration, and severity of any adverse event, and be responsible for evaluating and determining its relevance with vaccination. At the same time, it is necessary to record the measures taken, concomitant medication/treatment and non-pharmacotherapy in response to the adverse event, its outcome, whether it is a serious adverse event, and whether it results in study termination.

(3) Long-term safety observation

At 12 months after complete series, the investigator will collect and record the subject's SAEs and pregnancy-related events through telephone interview, as well as through the subject's spontaneous reporting. The investigator needs to follow up, record and report the collected SAEs and pregnancy-related events in accordance with the requirements for SAE reporting and pregnancy-related event reporting specified in the protocol.

11.3.2 Safety Observations

(1) Routine safety observations

Routine safety observations include all solicited and unsolicited medical events related or unrelated to vaccination that occurred during the observation period in the clinical trial.

Injection-site (local) adverse events: Pain, induration, redness, rash, swelling and pruritus at the injection site.

Non-injection-site (systemic) adverse events: Fever (axillary temperature), diarrhea, nausea, vomiting, headache, myalgia (non-injection site), joint pain, chills, fatigue/asthenia, rash (non-injection site), an acute allergic reaction.

Other events: Any medical events other than the above events, such as acute diseases, accidental injuries, etc.

(2) Observations on measurements of blood biochemistry, hematology, coagulation, and urinalysis

Before Dose 1 as well as on Days 1, 4, 7 and 28 after each dose, subjects enrolled in the phase Ib clinical trial will be subjected to collection of fasting venous blood (for laboratory measurement

of blood biochemistry, hematology, and coagulation) and urine samples (for laboratory measurement of urinalysis).

The investigator needs to interpret the test results related to blood biochemistry, hematology, coagulation, and urinalysis after immunization with reference to the normal ranges provided by the testing laboratory: if the test result is within the normal range, it will be recorded as "normal". If the test result is not within the normal range and exceeds 1.2 times the upper limit or lower limit of the normal range, the investigator will comprehensively evaluate whether the result is clinically significant based on the subject's condition. Any result evaluated as "not clinically significant (NCS)" may not be recorded as an adverse event; any result evaluated as "clinically significant (CS)" will be judged as "abnormal" and recorded as an adverse event (AE), for which the severity and relatedness will be further determined.

Blood biochemical indicators: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), creatine kinase (CK), lipase, serum creatinine (Scr), urea, C-reactive protein (CRP).

Hematological indicators: White blood cell (WBC) count, lymphocyte (LY) count and percentage, monocyte (MONO) count and percentage, neutrophil (NEUT) count and percentage, eosinophil (Eos) count and percentage, red blood cell (RBC) count, hemoglobin (Hgb), platelets (PLTs).

Coagulation indicators: Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB).

Urine indicators: Urine protein (PRO), urine glucose, erythrocytes.

11.3.3 Grading Criteria for Safety Observations

Injection-site and non-injection-site adverse events (Table 2, Table 3), and laboratory indicators/test items (Table 4, Table 5, Table 6) are interpreted according to the NMPA's *Guideline for Adverse Event Grading Criteria in Clinical Trial of Preventive Vaccines*. Intensity of adverse events or laboratory indicators not involved in the grading tables is evaluated according to Table 7.

Table 2 Grading of Injection-site (Local) Adverse Events

Symptom/Sign	Grade 1	Grade 2	Grade 3	Grade 4
Pain	Having no influence or a slight influence on limb activities	Influencing limb activities	Influencing daily life	Leading to loss of the basic self-care ability, or hospitalization
Induration*, swelling #	In a diameter of 2.5 - < 5 cm or an area of 6.25 - < 25 cm^2, and having no influence or a slight influence on daily life	In a diameter of 5 - < 10 cm or an area of 25 - < 100 cm^2, and have some influence on daily life	In a diameter of ≥ 10 cm or an area of ≥ 100 cm^2, or ulceration or secondary infection or phlebitis or sterile abscess or wound drainage, or severely influencing daily life	Abscess, exfoliative dermatitis, and dermis or deep tissue necrosis
Redness, rash** #	In a diameter of 2.5 - < 5 cm or an area of 6.25 - < 25 cm^2, and having no impact or having a slight impact on daily life	In a diameter of 5 - < 10 cm or an area of 25 - < 100 cm^2, and have some influence on daily life	In a diameter of ≥ 10 cm or an area of ≥ 100 cm^2, or ulceration or secondary infection or phlebitis or sterile abscess or wound drainage, or severely influencing daily life	Abscess, exfoliative dermatitis, and dermis or deep tissue necrosis

Pruritus	Injection-site pruritus which is relieved spontaneously or within 48h after treatment	Injection-site pruritus which is not relieved within 48h after treatment	Influencing daily life	NA

Note: * In addition to direct diameter measurement for grading evaluation, progression changes of the measurement results should also be recorded.

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

For evaluation and grading of induration, swelling, redness and rashes, the indicator with the higher grade should be used, based on the functional grades and practical measurement results.

Table 3 Grading of Non-Injection-Site (Systemic) Adverse Events

Symptom/Sign	Grade 1	Grade 2	Grade 3	Grade 4
Fever (axillary temperature)	37.3-＜38.0	38.0-＜38.5	38.5-＜39.5	≥39.5, persisting for more than 3 days
Diarrhea	Slight or transient, 3-4 times/d, stool with abnormal properties, or slight diarrhea persisting for not more than 1 week	Moderate or persistent, 5-7 times/d, stool with abnormal properties, or diarrhea > 1 week	> 7 times/d, stool with abnormal properties, or bloody diarrhea, orthostatic hypotension, electrolyte imbalance, and requiring intravenous fluid > 2 L	Hypotensive shock, requiring hospitalization
Nausea	Transient (< 24 h) or intermittent, while food intake basically normal	Persistent nausea, leading to decreased food intake (24-48 h)	Persistent nausea, leading to little food intake (> 48 h) or requiring intravenous fluid infusion	Life-threatening (such as hypotensive shock)
Vomiting	1-2 times/24h and having no influence on activities	3-5 times/24h or activities restricted	> 6 times within 24h or requiring intravenous fluid infusion	Hypotension requiring hospitalization or nutrition by other routes
Headache	Having no influence on daily activities, and requiring no treatment	Transient; slightly influencing daily activities, and possibly requiring treatment or intervention	Severely influencing daily activities, and requiring treatment or intervention	Refractory; requiring emergency treatment or hospitalization
Muscle pain (Non-injection site)	Having no influence on daily activities	Slightly influencing daily activities	Severe muscle pain, and severely influencing daily activities	Emergency treatment or hospitalization
Arthralgia	Mild pain; not interfering with the function	Moderate pain; requiring an analgesic drug and/or interfering with the function, but having no influence on daily activities	Severe pain: requiring an analgesic drug and/or pain may influence daily activities	Disabling pain

Chills	Mild sensation of cold; chattering of teeth	Moderate tremor of the entire body	Serious/Persistent tremor	Emergency treatment or hospitalization
Fatigue and weakness	Having no influence on daily activities	Influencing normal daily activities	Severely influencing daily activities and unable to work	Emergency treatment or hospitalization
Acute allergic reactions**	Local hives (blisters); requiring no treatment	Local hives, requiring treatment, or mild angioneurotic edema, requiring no treatment	Extensive hives or angioneurotic edema, requiring treatment, or mild bronchospasm	Allergic shock or life-threatening bronchospasm or laryngeal edema

** means Type I hypersensitivity reactions.

Table 4 Grading of Hematological Indicators

Test item (indicator)/Grading	Grade 1	Grade 2	Grade 3	Grade 4
Increased white blood cell count (WBC, 10^9/L)	11-＜13	13-＜15	15-＜30	≥ 30
Decreased white blood cell count (WBC, 10^9/L)	2.000-2.499	1.500-1.999	1.000-1.499	＜1.000
Decreased lymphocytes (LY, 10^9/L)	0.75-1.00	0.5-0.749	0.25-0.49	＜0.25
Decreased absolute neutrophil count (ANC, 10^9/L)	0.800-1.000	0.600-0.799	0.400-0.599	＜0.400
Eosinophils (Eos, 10^9/L)	0.65-1.5	1.51-5.0	＞5.0	Hypereosinophilic syndrome (HES)
Decreased platelet count (PLT, 10^9/L)	125-140	100-124	25-99	＜25
Low hemoglobin (g/dL) for men	10.0-10.9	9.0-＜10.0	7.0-＜9.0	＜7.0
Low hemoglobin (g/dL) for women	9.5-10.4	8.5-＜9.5	6.5-＜8.5	＜6.5

Table 5 Grading of Blood Biochemical Indicators

Test item (indicator)	Grade 1	Grade 2	Grade 3	Grade 4
Hepatic function (Increased ALT and AST)	1.25-＜2.5 × ULN	2.5-＜5.0 × ULN	5.0-＜10 × ULN	≥10 × ULN
Increased total bilirubin (mg/dL; μmol/L)	1.1-＜1.6 × ULN	1.6-＜2.6 × ULN	2.6-5.0 × ULN	≥5.0 × ULN
Creatine phosphokinase (CPK)	1.25-＜1.5 × ULN	1.5-＜3.0 × ULN	3.0-＜10 × ULN	≥10 × ULN
Pancreatic enzymes (amylase, lipase)	1.1-＜1.5 × ULN	1.5-＜3.0 × ULN	3.0-＜5.0 × ULN	≥5.0 × ULN

Note: ULN means the upper limit of normal range.

Table 6 Grading of Urine Indicators

Test item (indicator)	Grade 1	Grade 2	Grade 3	Grade 4
Urine protein (PRO) (urine dipstick test)	1+	2+	3+ or higher	NA
Urine glucose (urine dipstick test)	Minimal - 1+ Or ≤ 250 mg	2+ Or > 250 - ≤ 500 mg	> 2+ Or > 500 mg	NA

RBC (microscopic examination) [Red blood cell count per high power field (RBC/hpf) (except menstrual phase in females)]	6-＜10	≥ 10	Visible hematuria, accompanied or not accompanied by blood clot; or urine red blood cell cast; or requiring treatment	Emergency treatment or hospitalization

Table 7 Grading of the Intensity of Other Adverse Events

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Mild: Short-term (< 48h) or slight discomfort; having no influence on activities and requiring no treatment	Moderate: Mild or moderate activity restricted; possibly requiring a visit, and requiring no or mild treatment	Severe: Activities obviously restricted; requiring a visit and treatment, and possibly requiring hospitalization	Critically ill: Possibly threatening life; activities severely restricted; requiring intensive treatment	Death

11.3.4 Relevance of Adverse Events to the Investigational Vaccine

The investigator should have measures to determine in time the relevance of all adverse events/abnormal laboratory indicators to vaccination, so as to discover in time SAEs related to vaccination, cluster adverse events and adverse events with a tendency that occur in the clinical study, as well as suspend and terminate the clinical trial in a timely manner to minimize harm to subjects. Whether adverse events and abnormal laboratory indicators are related to vaccination and the degree of relevance are determined in accordance with the following principles.

Definitively unrelated: The subject has not used the investigational vaccine; or the adverse event occurs in an implausible time relationship to administration of the investigational vaccine; or there are other significant reasons that may result in the adverse event.

Unlikely related: There is evidence of administration of the investigational vaccine; the adverse event is more likely to be caused by other reasons; a negative or uncertain result is observed after re-administration of the investigational vaccine.

Possibly related: There is evidence of administration of the investigational vaccine; the adverse event occurs in a plausible time relationship to administration of the investigational vaccine; administration of the investigational vaccine cannot be ruled out as a cause of the adverse event, but other reasons may be the cause.

Probably related: There is evidence of administration of the investigational vaccine; the adverse event occurs in a plausible time relationship to administration of the investigational vaccine; the occurrence of the adverse event is explained by the investigational vaccine more reasonably than other reasons.

Definitely related: There is evidence of administration of the investigational vaccine; the adverse event occurs in a plausible time relationship to administration of the investigational vaccine; the occurrence of the adverse events is explained by the investigational vaccine more reasonably than other reasons; a positive result is observed after re-administration of the investigational vaccine; the adverse events are consistent with previous knowledge of this or this type of vaccine.

11.3.5 Adverse Event Outcomes

Adverse event outcomes include full recovery, improvement, stability, sequelae, and others (death, unknown).

11.4 Management of Biological Samples

11.4.1 Management of Samples for Hematology, Blood Biochemistry, Coagulation and Urinalysis

Subjects' blood and urine samples collected before and after each dose will be stored at room temperature and protected from direct sunlight, transported by designated personnel to the clinical laboratory for hematology, blood biochemistry, coagulation, and urinalysis.

11.5 Immunogenicity Observation Method

- Subjects included in the specific immunity subgroup:

1) Before Dose 1, about 4.0 mL/tube of blood (20.0 mL in total) will be collected in five anticoagulant tubes (heparin sodium) for exploratory observation of the percentage of specific immune cell subsets, the cytokine level, and memory T cells.

2) At 7 days after Dose 1 and at 7 days & 15 days after complete series, about 4.0 mL/tube of blood (16.0 ml in total) will be collected in four anticoagulant tubes (heparin sodium) for exploratory observation of the percentage of specific immune cell subsets, and the cytokine level.

3) At 28 days after complete series, about 4.0 mL/tube of blood (4.0 mL in total) will be collected in one anticoagulant tube (heparin sodium) for exploratory observation of memory T cells.

4) If the subjects are included for immune persistence evaluation, see "Subjects in the recommended-dose study group" below.

- Other subjects:

1) Before Dose 1, at 28 days after Dose 1 and at 7, 15 & 28 days after complete series, blood will be collected for measurement of neutralizing antibody and specific IgG antibody.

2) If the subjects are included for immune persistence evaluation, see "Subjects in the recommended-dose study group" below.

- Subjects in the recommended-dose study group:

In addition to above measurement of humoral immunity or specific cellular immunity, observation of immune persistence within 3-12 months after complete series will also be required.

1) At 3 months after complete series, about 6.0 mL of venous blood will be collected and placed in a coagulation tube (only contains a coagulant) for measurement of neutralizing antibody and specific IgG antibody; in addition, for subjects included in the specific cellular immunogenicity subgroup of the recommended-dose study group, about 4.0 mL/tube of blood (4.0 mL in total) will be collected in an anticoagulant tube (heparin sodium) at 3 months after complete series, for exploratory observation of the percentage of memory T cells.

2) At 6 months after complete series, about 6.0 mL of venous blood will be collected and placed in a coagulation tube (only contains a coagulant) for measurement of neutralizing antibody and specific IgG antibody; in addition, for subjects included in the specific cellular immunogenicity subgroup of the recommended-dose study group, about 4.0 mL/tube of blood (20.0 mL in total) will be collected in five anticoagulant tubes (heparin sodium) at 6 months after complete series, for exploratory observation of the percentage of specific immune cell subsets, the cytokine level, and memory T cells.

3) At 9 and 12 months after complete series, about 6.0 mL of venous blood will be collected and placed in a coagulation tube (only contains a coagulant) for measurement of neutralizing antibody and specific IgG antibody.

11.6 Management of Samples for Immunogenicity Testing

(1) Handling, Storage and Transportation of Blood Samples

Humoral immunity test sample: After venous blood is collected, the blood sample will be fully coagulated, and its serum will be separated by centrifugation within 24 hours. If it cannot be centrifuged on the day, it needs to be stored in a refrigerator at 2 ℃-8 ℃ and the storage temperature should be recorded. Separated serum will be aliquoted into 3 test cryogenic vials and 1 backup cryogenic vial. The 3 test vials will be sent to the National Institutes for Food and Drug Control (NIFDC), China for testing. Each test tube contains ≥ 0.5 mL of serum, and the remaining serum will be stored in the backup cryogenic vial. Test serum and backup serum will be stored separately in different low-temperature refrigerators/equipment at -20 ℃ or below, which will be locked and managed by designated personnel, and the temperature will be recorded in accordance with the SOP. During the transportation of serum samples, the transport temperature should be kept at <-20 ℃. During the storage and transportation of serum, if the temperature is ＞-20 ℃ and ＜0 ℃, the cold chain will be determined abnormal, and if the temperature is ≥0°C, the cold chain will be determined broken.

Blood samples for measurement of specific cellular immunity as an exploratory endpoint: After heparin sodium-anticoagulated venous blood is collected, it will be placed in a refrigerator at 2 ℃-8° 8 for storage and the storage temperature will be recorded, and it should be sent for testing as soon as possible.

(2) Numbering rule for blood samples

All subjects and subjects in the recommended-dose study group will be subjected to blood sampling for observation of immune persistence. Each sample corresponds to 5 blood sample labels, of which, 1 large label will be attached to a blood collection tube, and 4 small labels will be respectively attached to serum aliquot tubes: Test Serum Tube A (IgG test), Test Serum Tube B (pseudovirus neutralizing test), Test Serum Tube C (wild-type virus neutralizing test), and Backup Serum Tube D. The numbering rule for blood samples is "Study subject number-Time after Dose 1-Letter code for test serum", for example "001-D0-A" denotes Test Serum A separated on the day of vaccination from Subject 001.

For subjects included in the specific cellular immunogenicity subgroup, each sample collected before Dose 1 or at 6 months after complete series will be required to correspond to 5 small blood sample labels, which are respectively attached to Blood Collection Tubes E, F, G, H and I for cellular immunity tests; each sample collected at 7 days after Dose 1 or at 7 days/15 days after complete series will be required to correspond to 4 small blood sample labels, which are respectively attached to Blood Collection Tubes E, F, G and H for cellular immunity tests; each sample collected at 28 days or 3 months after complete series will be required to correspond to 1 small blood sample label, which is attached to Blood Collection Tube I for cellular immunity tests; the numbering rule for blood samples is "Study subject number-Time after Dose 1-Letter code for test serum".

(3) Serum sample testing

Serum samples for humoral immunity tests will be tested by the entrusted facility National Institutes for Food and Drug Control (NIFDC), China. The level of specific IgG will be measured by ELISA; the level of neutralizing antibody will be measured by the pseudovirus neutralizing test

and wild-type virus neutralizing test, respectively; the reference standard and quality control standard will be provided by the testing laboratory.

Blood samples for measurement of specific cellular immunity as an exploratory endpoint will be sent to the laboratory designated by Tsinghua University. Each test item (indicator) will be measured by flow cytometry and/or the interferon-γ enzyme-linked immunospot (ELISPOT); the reference standard and quality control standard will be provided by the testing laboratory.

11.7Data Management

Data management plan: The data administrator will develop a data management plan based on the clinical trial protocol, and implement it after review and approval by the sponsor.

Database establishment: The eCRF will be designed by the data administrator according to the clinical trial protocol, and the Electronic Data Capture (EDC) system will be built by the database builder. The data administrator will write a data validation plan (DVP) based on the protocol and eCRF, and the database builder should set up edit checks based on the DVP. User Acceptance Testing (UAT) will be performed prior to the use of the EDC system, and a test report will be written. The eCRF system that has passed a test cannot be put into operation before it is reviewed and approved by the sponsor.

Data entry: The data administrator will develop a data entry guideline and train the investigator and/or EDC entry personnel, and then the investigator and/or EDC entry personnel will enter data into the EDC system.

Permission assignment (PA): After the EDC system is operated, the system administrator will create accounts and grant different permissions based on training records and account application status.

Data queries: The data administrator will develop a Data Validation Plan (DVP) and implements it after approval by the sponsor. When data are entered into the EDC system, it will verify the data according to the established logic (Edit Check) in the data validation plan, and give a system question for any suspected data; for data that cannot be set as a mode in which a question is given by the system, an artificial question will be given by the EDC system. Entry personnel or the investigator will confirm and answer the artificial question and the system question, until the question is solved. If the answer cannot solve the question, then the data administrator and the clinical research associate may propose a second question. All traces will be stored in the EDC database.

External data: Based on the study protocol and its implementation, the data administrator will develop an external data transmission protocol, which will take effect after the sponsor signs and approves it. The laboratory or the sponsor will perform data transmission in accordance with the format, time and frequency specified by the external data transmission protocol. The data administrator will conduct a consistency check, and the data in question will be notified to the sponsor by email. The corresponding data will be transmitted again after each party revise them or verify that they are correct, until the relevant data remains consistent.

Medical coding: The data management organization will be responsible for medical coding in this study. Adverse events in the clinical trial will be coded using the MedDRA specified in the DMP.

Data review: After completion of data cleanup, question answering (QA) and database closure (before locking), all relevant parties (the sponsor, investigator, data manager, statistician) will review the database undergoing data clarification and biological sample test results. The *Data Review Report* and *Resolution on Population Division* will also be finalized.

Database locking: After data are verified without errors, the database will be locked with written approval by relevant personnel (the statistician, investigator, and sponsor) via joint signature (name and date). Database unlocking and re-locking shall not be carried out without the written consent from the above relevant personnel, and records should be made. After the database is locked, the data administrator needs to submit the locked database to the statistician for statistical analysis.

Data management report: The data administrator will write a data management report after the database is locked.

11.8 Statistical Analysis

11.8.1 Analysis Sets

Full Analysis Set (FAS): Based on the intention-to-treat (ITT) principle, all randomly allocated subjects who have received at least one dose and undergone pre-immunization blood sample collection with valid antibody titers are included. Subjects vaccinated with wrong experimental vaccine will be included in the group based on randomization results for immunogenicity assessment as per the ITT principle.

Per Protocol Set (PPS): It includes all randomly allocated subjects who are consistent with the inclusion criteria/inconsistent with exclusion criteria, and have completed a full course of immunization and pre-immunization & post-immunization blood sample collection for immunogenicity assessment as per the protocol, with valid antibody titers. Subjects who meet the following conditions will be reviewed at a blind data review meeting to decide whether they may be included in the PPS:

- Those who violate the study protocol.
- Those who are mistakenly vaccinated.
- Those who have used vaccines or drugs contraindicated in the protocol.
- Other situations that may affect immunogenicity assessment.

In this study, the above FAS and PPS are respectively defined at 28 days after Dose 1 and at 7 days, 15 days and 28 days after complete series and are mainly used for immunogenicity assessment.

Safety Set (SS): It includes all randomly allocated subjects who have received at least one dose. According to the ASaT (All Subjects as Treated) principle, safety evaluation will be performed for subjects mistakenly vaccinated based on the actual vaccination.

In this study, safety analysis will be performed based on the number of subjects actually vaccinated for each dose, i.e., Safety Set for Dose 1, denoted by SS1, includes all the subjects receiving Dose 1; the safety set for Dose 2, denoted by SS2, includes all the subjects receiving Dose 2.

Immunogenicity Persistence Set (IPS): It includes all subjects who have undergone blood sample collection at the time points for immune persistence assessment and have valid data on immunogenicity assessment. IPS is defined for each time point (3, 6, 9 and 12 months after a full course of immunization) for immune persistence assessment.

The above analysis sets will be discussed and determined jointly by the investigator, the sponsor, the statistical party, and the data management party at a data review meeting prior to database locking.

11.8.2 Statistical Analysis Methods

11.8.2.1 General Principle

Measurement data will be statistically described by the mean, median, standard deviation, maximum, and minimum; count/ordinal data will be expressed by the count and frequency.

Statistical analysis will be performed using SAS 9.4 or a higher version.

11.8.2.2 Distribution, Demographic Information and Baseline Characteristics of Subjects

The numbers of subjects that are screened, enrolled, and complete the study will be summarized, as well as the numbers of subjects in the analysis sets; the reasons for the dropout of dropout subjects will be analyzed. The demographic characteristics and baseline characteristics of subjects will be statistically described.

11.8.2.3 Immunogenicity Analysis

The seroconversion rate and positive rate of neutralizing antibody after immunization in each group will be calculated with the two-sided 95% confidence intervals by using the Clopper-Pearson method; statistical analysis will be performed by using the Chi-square test/Fisher's exact test.

The geometric mean titer (GMT), and geometric mean fold increase (GMI) of neutralizing antibody after immunization in each group will be calculated by using the geometric mean and the 95% confidence interval; the intergroup differences will be statistically tested by using the log-transformed analysis of variance.

The IgG antibody after immunization will be analyzed by using the same statistical method for analysis of neutralizing antibody.

The percentages of $CD4^+$, $CD8^+$, $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}2^+$, $CD4^+TNF\alpha^+$, $CD4^+IL\text{-}4^+$, $CD4^+IL\text{-}13^+$, $CD8^+IFN\text{-}\gamma^+$, $CD8^+IL\text{-}2^+$ and $CD8^+TNF\alpha^+$ as cell subsets specific to the receptor-binding domain (RBD) of the spike (S) protein, the levels of IFN-γ and IL-2 as cytokines specific to the RBD of the S protein, and the percentages of $CCR7^+CD45RA^-CD4^+$ and $CCR7^+CD45RA^-CD8^+$ as memory T cells will be statistically described; intergroup differences will be statistically analyzed using non-parametric tests.

11.8.2.4 Safety Analysis

Adverse events will be coded using the MedDRA. In this study, statistical analysis will be conducted mainly for the adverse events that occur after vaccination, and the adverse events that occur before vaccination will be listed.

The frequency, number of subjects and the incidence of all adverse events, adverse events related to the investigational vaccine, and adverse events unrelated to the investigational vaccine in each group will be calculated; statistical analysis of intergroup differences will be performed by using the Fisher's exact test. Statistical analysis of the severity of adverse events and vaccine-related adverse events, the numbers of doses resulting in these events, and the time of their occurrence will be performed. Adverse events related to the investigational vaccine, and adverse events unrelated to the investigational vaccine will be performed. Statistical analysis of adverse events following each dose will also be performed. Adverse events following each dose will be analyzed based on the Safety Set for each dose.

The frequency, number of subjects and the incidence of all serious adverse events, serious adverse events related to the investigational vaccine, and serious adverse events unrelated to the investigational vaccine in each group will be calculated; statistical analysis of intergroup

differences will be performed by using the Fisher's exact test. All serious adverse events will be listed.

The changes in hematological indicators, blood biochemical indicators and coagulation indicators after vaccination will be statistically described; a crosstab will be used to statistically describe the clinically significant (CS) changes in hematological indicators, blood biochemical indicators, urine indicators and coagulation indicators after vaccination.

11.8.2.5 Processing of Missing Data

In the statistical analysis for the FAS, imputation will be performed using Last Observation Carried Forward (LOCF) for the subjects with missing neutralizing antibody test results. In this study, missing data on the cellular immunity and safety endpoints will not be processed.

12 Safety of Subjects and Management of Adverse Events

12.1 Definitions of Adverse Events

Adverse Event (AE): Any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participants' involvement in the research, whether or not considered related to participation in the research.

Adverse Vaccine Reaction (AVR): Any noxious or undesirable reaction in a clinical trial that might be related to the investigational vaccine. There is at least a reasonable probability for a causal relationship between the investigational vaccine and the adverse reaction, that is, the relevance cannot be ruled out.

Serious Adverse Event (SAE): Any adverse event that results in death, life-threatening, or places the participant at immediate risk of death from the event as it occurred, requires or prolongs hospitalization, causes persistent or significant disability or incapacity, results in congenital anomalies or congenital disabilities, another condition which investigators judge to represent significant hazards.

Suspected Unexpected Serious Adverse Reaction (SUSAR): It refers to any suspected and unexpected serious adverse reaction whose nature and severity are not consistent with the existing information such as the investigator's brochure on the investigational vaccine/drug, the instruction for use of the marketed vaccine/drug, or product characteristic summary.

Adverse Event Following Immunization (AEFI): Any untoward medical occurrence which follows immunization and which does not necessarily have a causal relationship with the usage of the vaccine.

12.2 Safety Assurance

The clinical trial shall be conducted in a municipal-level hospital that is qualified to carry out preventative immunization. Before the clinical trial, the sponsor shall assess the clinical trial center in accordance with the GCP, including inspecting whether the environment and facilities of the clinical research center conform to the requirements of the *Guiding Principles for Quality Management of Vaccine Clinic Trials (For Trial Implementation)*, whether the first-aid facilities and first-aid equipment in the first-aid room are effective, whether the first-aid medicine is in the validity period, and whether the first-aid doctor has acquired corresponding qualifications and competence. Where a subject experiences an AE at the clinical trial center, the department shall timely take actions for treatment at the first-aid room of the clinical trial center, and arrange for follow-up hospitalization. Whether the clinical trial department and the hospital enter into the *Green Channel Process*, the subject, once enrolled, shall notify the department to make preparation

for timely treatment. There shall be a strict SOP, which provides such measures as responsibilities of professionals, telephone numbers and rescue route to assure that an emergency AE will be handled timely. Effective communication between the subject and the investigator shall be maintained to assure that any AE will be reported and handled promptly. Where a subject experiences an SAE, which necessitates hospitalization for emergency treatment, the department shall be able to provide such green channel services as providing medical advice, hospitalization and medical protection to assure that the subject will receive timely treatment.

The sponsor shall designate full-time staff to be responsible for the management of clinical trial safety information monitoring and SAE reporting. The sponsor and the investigator shall formulate standard operating procedures for clinical trial safety information monitoring and SAE reporting, and providing trainings for all the personnel concerned. AE monitoring and reporting for the vaccine in clinical trial shall be jointly completed by the subjects, the AE investigators and the investigator at different stages and different observation time points.

12.3Detection and Collection of AEs

When providing the subject with training, the investigator shall emphasize the need for timely reporting of AEs. The investigator shall attach high importance to the adverse events, and timely investigate and handle the same.

Where the subject experiences solicited AEs and unsolicited AEs, the investigator shall ask the subject whether he/she receives such treatment as hospitalization and outpatient service for any reason, or self-administered medication and record the above information.

Where an SAE occurs, the investigator shall be obligated to review all the documents related to such event (e.g., course of disease and medical advice records, laboratory report and diagnosis report), or shall arrange for clinical inspection/test as required by the sponsor, for the purpose of clarifying the SAE's nature and relatedness. Where a subject is confirmed to suffer death in the course of the clinical trial or during the follow-up period, the investigator shall collect the hospital's conclusion about the deceased. Where an autopsy is performed, the investigator shall obtain a copy of the results, including histopathological results.

The investigator shall make his/her best efforts to collect a complete case copy. Instead of substituting the subject's case copy for the trial record, the investigator shall record all the information related to the SAE in the original record, eCRF and the serious adverse event report sheet.

Where the medical record shall be disclosed for medical identification, all the content columns which render the subject's identity identifiable shall be covered prior to the disclosure.

12.4Treatment and Management of AEs

1. The principles of handling fever reactions suffered by a subject administered the vaccine in the course of the clinical trial mainly include the following:

1) Where the subject's body temperature is less than 38.5℃, and the subject can tolerate the fever reaction, he/she shall receive physical cooling; where the subject is unable to tolerate the fever reaction, he/she shall receive an oral administration of paracetamol, and be encouraged to drink water as much as he/she can.

2) Where the subject's body temperature is equal to or more than 38.5, he/she shall receive an oral administration of paracetamol, and the physical condition of the subjects was observed closely.

3) Where the subject’s body temperature is equal to or more than 38.5℃, and the fever reaction lasts for more than 48h, he/she shall receive medical treatment and be closely observed the subject's physical condition. Where the drugs above yield no significant results, the investigator shall consult the physicians for treatment.

2. If a subject develops AE during the trial, the investigator and specialist should monitor the subject closely for changes in AE and provide appropriate treatment if necessary

The investigator shall formulate an emergency response plan for SAE management in the clinical trial, and train all the personnel concerned accordingly. The investigator shall take actions to gain timely access to any clinically significant disease/event that occurs after the vaccination, and ensure that the subject timely receives appropriate treatment in the designated hospital according to the national regulations. Drugs used for treating the AE shall be recorded in the subject’s Source Document and eCRF.

Where any difference or dispute arises from the handling of AEs, the investigator shall be obligated to assist the sponsor in dealing with the same, and assist the subject with the medical appraisal.

The sponsor shall be obligated to unconditionally protect the safety of the subject. For any adverse reaction that is confirmed to be related to the vaccine, the sponsor shall compensate the subject as required by the *Vaccine Administration Law of the People's Republic of China*, the *Regulation on the Administration of Circulation and Vaccination of Vaccines*, and the *Regulation on the Compensation for Adverse Reactions of Immunization (for Trial Implementation)* at the provincial level.

The investigator shall pay continuous attention to any AE that survives the end of the clinical trial or the conclusion of the follow-up visit, and shall follow up the AE related to immunization till the event ends. Where any unrelated event such as disease is diagnosed by the doctor, the investigator may cease the follow-up visit procedure.

12.5SAE Reporting

12.5.1 Reporting Procedures

The investigator shall, after becoming aware of an SAE, fill out the first report on the Serious Adverse Event Report Form within 24 hours, and submit it to the sponsor and the ethics committee of the responsible institution via facsimile, email, or online report. The investigator shall, after completing the first report, follow up the SAE, timely submit new information or information about amendments to the previous report and the prognosis of the event in the form of follow-up visit procedure/summary report, pay attention to the SAE in which the subject is not recovered or being recovered until the subject is recovered/stabilized, and prepare a summary report. Where the report involves an event of death, the investigator shall provide the sponsor and the ethics committee with other necessary information, such as the autopsy report and final medical report.

Upon receiving the safety information related to the clinical trial from the sponsor, the investigator shall timely sign the receipt thereof, read the safety information, and consider whether the treatment of the subject is adjusted accordingly. If necessary, the investigator shall communicate with the subject as early as possible, and report any SUSAR provided by the sponsor to the ethics committee.

The sponsor shall, after being informed of an SUSAR, promptly conduct a comprehensive analysis, assessment, and judgement of the serious adverse event. The sponsor shall submit the first report to all the investigators participating in the clinical trial, the clinical trial institution, the ethics committee, the food and drug administration department and the competent health

department according to the following time limits: (I) The sponsor shall, after being informed of any SUSAR that results in death or is life-threatening, promptly report the case not later than 7 natural days, and report relevant follow-up information within 8 natural days thereafter. (II) The sponsor shall, after being informed of any SUSAR that does not result in death or is not life-threatening, promptly report the case not later than 15 natural days. (III) The sponsor shall, after being informed of other potential serious safety risks, shall promptly report the case not later than 15 natural days.

The sponsor shall be obligated to protect the safety of the subject, give humane care for any subject experiencing an AE in the course of the clinical trial, and treat and compensate any subject experiencing an AE that is definitely related to immunization. See the Safety Management Plan for details.

12.5.2 Reporting Content

- Type of report and reporting time.
- Information about the subject.
- Information about the reporter.
- Information about the investigational vaccine.
- Information related to the clinical trial.
- Information about comorbidity and treatment.
- Detailed information about the SAE.
- Time when the investigator is informed of the SAE.
- Signature of the investigator.

13 Quality Assurance and Quality Control in Clinical Trials

13.1 Investigator

The study sites of the vaccine clinical trial shall formulate a unified standard operating procedure prior to the initiation of the clinical trial, and strictly execute the standard operating procedure to safeguard the quality control of the clinical trial.

Prior to the clinical trial, the following staffs shall be designated for the study sites of the vaccine clinical trial: principal investigator, quality controller for the clinical trial.

All staffs participating in the clinical trial shall receive corresponding training, obtain authorization from the principal investigator, and be aware of their responsibilities before involving in the actual work of implementing the clinical trial.

The study sites of the vaccine clinical trial shall carry out quality control activities as appropriate, inspect on the work of each relevant party, timely identify problems and risks, and follow up the problems identified during the inspection till they are solved, so as to ensure the clinical trial conforms to the requirements prescribed in the GCP and the protocol.

13.2Sponsor

The sponsor is ultimately responsible for the quality of the clinical trial. The sponsor shall establish a well-developed vaccine clinical trial quality management system, formulate corresponding SOPs, organize an inspection of the clinical trial, and conduct a systematic inspection on the activities and documents related to the clinical trial, as well as on the study sites, laboratories and the CRO, so as to evaluate whether the clinical trial is carried out in accordance with the clinical trial protocol, SOPs and relevant regulations, and whether the trial data is recorded in a timely, true, accurate and complete manner.

Prior to the clinical trial, the sponsor shall conduct a comprehensive assessment for the study sites pursuant to the implementation conditions and requirements for clinical trial, and select the study sites based on the results of the assessment.

The sponsor shall organize a supervision on the clinical trial, and appoint a sufficient number of monitors to supervise the clinical trial from throughout the whole course of the study.

The sponsor shall designate a full-time staff to be responsible for the management of safety information monitoring and SAE reporting in the clinical trial, staying up to date for the clinical trial safety information, and timely reporting to all investigators participating in the clinical trial and regulatory authorities.

The sponsor shall participate in the investigation and disposal of adverse reactions/events, and take responsibility for providing medical attentions or otherwise compensation to any subject who experiences an adverse reaction or an adverse event that is clinically related to the vaccine.

The sponsor shall organize quality control activities in the course of clinical trial, so as to improve the quality of clinical trial.

The sponsor may, taking into account the actual status of the clinical trial, decide on whether it is necessary to organize an inspection on the clinical trial to guarantee its quality, so as to assure that the clinical trial is carried out in accordance with the requirements prescribed in the GCP and the protocol.

13.3Monitor

The number of monitors shall be sufficient to fulfill work demands. The monitor shall have the education background and work experience of medicine, pharmacy, or any other relevant discipline.

The monitor shall be responsible for overseeing and inspecting the whole process of the clinical trial, to ensure the clinical trial process conforms to the requirements prescribed in the GCP and the clinical trial protocol. The monitor shall take the following responsibilities:

- Before the trial, confirm whether the institutions responsible for the clinical trial meet relevant requirements, including the equipment of qualified staffs and the implementation of proper trainings, as well as whether the laboratories are fully equipped, well-functioning and qualified regarding the requirements of the study.
- Provide all the staff involved in the clinical trial with corresponding guidance and training, and ensure the investigator is familiar with the requirements prescribed in the trial protocol, the operating procedures for each link and relevant regulations.
- Verify that the qualification and authorization of the investigators involved in the trial conform to requirements.
- Verify whether the investigators follow the trial protocol, SOPs and relevant regulations in the course of the clinical trial.
- Ensure that each subject has signed the informed consent form prior to participate in the trial, and confirm that subjects enrolled are eligible.
- Acquire the progress of the trial and report the same to the sponsor and the investigator.
- Ensure that all the trial information of the subjects is recorded in the source document in an accurate, timely, complete, and regular manner; the information is entered into EDC correctly and is consistent with the source data; all errors or questions have been corrected or clarified.
- Ensure that all adverse events are recorded as well as that serious adverse events are reported and recorded within the specified time.
- Ensure that all the events that lead to a violation of or deviation from the protocol are timely and accurately recorded, as well as reported as required.
- Verify whether the investigational vaccine is supplied, stored, distributed, used, and returned in accordance with relevant regulations, and ensure that the above procedures are recorded accordingly.
- Verify whether the biological samples are collected and stored in accordance with the protocol and relevant SOPs, and ensure that the above procedures are recorded accordingly.
- Assist the investigator in handling the procedures for necessary notification and application, and report the data and results of the clinical trial to the sponsor.
- Conduct periodic supervision on the study sites and submit a supervision report to the sponsor.

13.4 Blood Sample Management

- Blood sample collection: The process of blood collection shall comply with the provisions of relevant SOPs. Blood collection shall be recorded. The blood collection tubes shall be identified in an easy and unique manner.
- Serum separation: After the subject's blood sample is fully coagulated, the serum shall be timely separated (within 24 hours) and recorded. The separated serum shall be divided into serums for testing and back-up serums. The serum tubes shall be identified in an easy, unique, and traceable manner.
- Serum cold-chain management: Serum samples shall be managed by specialized personnel. The storage log for serum samples shall be established. Serums for testing and back-up serums shall be separately stored in different cryogenic refrigerators/equipment at -20℃ or below. The blood sample administrator shall monitor the serum storage temperature punctually in accordance with the blood sample management SOPs or relevant regulations and the storage temperature should be recorded to avoid the occurrence of temperature violation. In the event of temperature violation, the blood sample administrator shall timely report to the sponsor. Where the temperature in the course of serum storage and transportation is higher than -20℃ and lower than 0℃, the cold chain shall be considered abnormal; where the temperature is equal to or higher than 0℃, the cold chain shall be considered damaged.
- Submission of serum for testing: Prior to submission for testing, the investigator shall organize the serums for testing as required by the testing institution. The temperature in the course of serum transportation shall be kept at -20℃ or below.
- Storage and disposal of back-up serums: Back-up serums shall be properly stored by the investigator and managed based on the blood sample management SOPs or relevant regulations. The study sites shall not dispose of the back-up serums until the sponsor puts forward a proposal.

13.5 Vaccine Management

- Supply of vaccines: The sponsor shall provide the investigator with vaccines with easily identified, correctly coded labels on which "for clinical trial only" is noted.
- Vaccine transportation: The whole process of vaccine management shall conform to the cold-chain requirement. Proper conditions for vaccine transportation and storage as required by the protocol should be ensured. Complete records of the transportation and temperature monitoring should be provided.
- Hand-over of vaccines: Upon receiving vaccines, the vaccine administrator shall verify the lot number, amount, validity period and transportation status of the vaccines, as well as timely fill in the vaccine hand-over record.
- Cold-chain management of vaccines: The vaccine counter should be locked and managed by a specially assigned person. Experimental vaccines shall be placed in a separate area with clear identification. Vaccines shall be stored away from light under 2℃-8℃. A vaccine accountability log shall be established by the vaccine administrator, and the vaccine storage temperature shall be punctually monitored in accordance with the vaccine management SOPs or relevant regulations with records made to avoid the occurrence of temperature violation. In the event of temperature violation, the investigator shall take measures to respond and report to the sponsor timely.

- Distribution and use of vaccines: The distribution and use of vaccines shall be recorded in detail. The immunization process should be traceable, including the date when the vaccine is distributed to the subject, the distributor and information about the vaccine being distributed for immunization (e.g., vaccine number). The temperature in the course of vaccine use shall be recorded as required by corresponding SOPs. The investigator shall assure that the vaccine is administered within the validity period, and that the vaccine is not administered to people other than the subjects included in the study.
- Collection and inventory of vaccines: The vaccine administrator shall timely collect remaining vaccines, conduct periodic inventory, and retain the inventory records, so as to ensure that the sum of used and remaining vaccines matches the total.
- Disposal of remaining vaccines: Discarded, expired or remaining vaccines shall be stored according to relevant SOPs or relevant requirements. The study sites shall not destroy such vaccines until the sponsor puts forward a proposal for their disposal.
- Collection and handling of empty vaccine boxes/bottles: After use, empty vaccine boxes/bottles shall be timely collected, sorted, and counted by designated personnel. A accountability log for the management of empty box/bottle shall be established to ensure the number of empty boxes/bottles is consistent with the number of used vaccines. The study sites shall not destroy the empty boxes/bottles until the sponsor puts forward a proposal for their disposal.

13.6 Data Management

According to the requirements of the GCP and the provisions of this protocol, the clinical trial process shall be recorded in detail. Where the original record shall be corrected, such correction shall only be made by marking off and adding a sidenote to specify the corrected data. The investigator shall sign beside the correction and specify its date. The original record shall not be erased or covered. The Source Document, the Diary Card and the Contact Card shall truly record the whole process of the subject participating in the clinical trial, as those are important data that ensures the traceability of the clinical trial, which shall be kept intact. Any observation/detection result and AE verification in the course of clinical trial shall be timely, accurately, completely, regularly and truly recorded in the Source Document, the Diary Card and the Contact Card. The authorized investigator shall complete the electronic transcription of necessary data.

In this clinical trial, the Electronic Data Capture (EDC) system will be used for collecting and managing clinical trial data. The data management party shall designate staff for database establishment to fulfill the establishment of EDC system and the responsibility of user management.

The data administrator and the monitor shall conduct periodic inspections on data records as required by the GCP. Any queries about data shall be reported to the investigator. The investigator shall timely make clarifications and revisions, based on which the data administrator and the monitor shall confirm the data records. If necessary, further queries may be raised to ensure that the data is true, reliable, and complete. All the inspection, queries, revision, and reply mentioned above shall be recorded in the EDC system and traces of inspections should be open for review and exportation.

After completing the entry, query and revision of the clinical trial data, audition for the blind data, and determining the analytic data set, the principal investigator, the sponsor, the statistical analysis personnel, the data administrator, the project manager and the clinical monitor representative shall lock the data, and prepare a database locking record. Where there is a need to

unlock the locked database, the data administrator shall, with the approval from the principal investigator, the sponsor, the data administrator, the statistical analysis personnel, the project manager and the clinical monitor representative after joint discussion, complete the revision of the database before re-locking the database according to the database locking procedure.

Data management plan: The data administrators designated by the data management party and the sponsor shall jointly compile a data management plan based on the clinical trial protocol and the setup status of the EDC system. The completed plan shall not be executed until it is examined and approved by the sponsor.

After the query about the database is clarified, the latest version of MedDRA in force at the time of the clinical trial shall be adopted to code the AEs.

The testing party shall hand over the immunogenicity database to the sponsor in the form of official documents. The data management party shall organize staffs to complete double verification.

After data management ends, the data administrator designated by the data management party shall compose the Data Management Report, which shall go into effect after the sponsor reviews, confirms and signs on the same.

13.7 Trial Data

The names and categories of documents which are to be kept by the investigator and the sponsor during the course of the clinical trial can be referred to in Appendix 2-Clinical Trial Storage Documents of the GCP.

- Data that contains identical information about the subjects (e.g., the Informed Consent Form, the Source Document) shall be kept at the study sites.

- Archive management shall be carried out pursuant to the SOPs or the archive room management rules; corresponding accountability logs shall be established; safety measures shall be taken to prevent pest, moisture, fire, theft and rodents.

- The use and review of the clinical trial data shall be limited to the personnel involved in the project within the study sites, personnel involved in the CRO project, the relevant personnel designated by the sponsor and auditors designated by the NMPA. All the data shall at least be kept till 5 years after the investigational drug is approved for license. The sponsor shall be notified of the expiration of the 5-year period. Without a prior written notice from the sponsor, no person shall be permitted to dispose of the data without permission.

13.8 Release of Clinical Trial Results

After the clinical trial ends, the investigator may, with the written consent and authorization from the sponsor, introduce, release or announce the methods and results of the clinical trial, in such manner as the sponsor approves, at workshops or national/regional professional conferences, or in journals, papers and academic lectures, provided that such introduction, release or announcement shall not infringe on the sponsor’s title and intellectual property right to the methods, results, data, reports, data and documents of the clinical trial. Clinical trial results that are negative or inconclusive shall be released or made public as if the results are positive.

14 Ethics Committee

14.1 Review of Clinical Trial

The ethics committee will review and approve the clinical trial protocol, the informed consent form, the investigator's qualification, relevant study forms, and grant permission.

-Drug Clinical Trial Approval issued by NMPA

-Certificate of Analysis of the investigational vaccine issued by the National Institutes for Food and Drug Control

-Clinical trial entrustment contract

-Confidentiality agreement

-Investigator's brochure

-Clinical trial protocol

-Informed Consent Form

-Notice of enrollment

-Adverse event record forms (e.g., the *Source Document*, the *Diary Card*, the *Contact Card*, eCRF)

-Other documents and materials.

14.2 Supervisions

14.2.1 Informed Consent

The enrollment method and relevant information provided to the subjects should be verified as complete and understandable; the approach of obtaining informed consent from the subjects should be determined proper. Throughout the course of the study, the ethics committee shall supervise the existence of ethical problems that are detrimental to the subjects and whether the subjects receive treatment, compensation, or corresponding insurance measures if he or she is harmed by his or her participation in the study. The participants will also be assessed for the degree of risk they might undertake.

14.2.2 Confidentiality

Prior to the initiation of the clinical trial, all the participating parties shall sign a confidentiality agreement to ensure that, within the scope permitted by existing laws or regulations, records and information concerning the subject's personal identity will be kept confidential during the process of study implementation, biological sample collection, as well as reporting and publication, and that such records and information shall not be made public or disclosed. Biological samples shall only be identified with the sample number or laboratory number. Only the principal investigator and the sponsor shall be granted access to electronic or written copies of materials concerning the subjects' identities.

14.2.3 Potential Harms and Minimization of Harms

Timely treatment should be provided in accordance relevant provisions if an adverse event is determined as related to vaccination. In case that any life-threatening events are identified, measures should be taken to ensure the subjects are sent to the hospital for treatment the events are reported in time.

Measures should be taken to ensure the venous blood samples are collected by trained and experienced medical staffs in accordance with prespecified procedure under supervision so as to minimize the pain experienced by the subject who undergoes blood collection.

15 References

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Annex 1 Summary of Revisions to the Clinical Trial Protocol

Section revised	Original text	Revised text	Reason for revision

Annex 2 Responsible Institution——Shulan (Hangzhou) Hospital

There are currently 10 full-time medical technicians in the phase I clinical trial laboratory, including 2 doctoral candidates, 3 master candidates, and 3 persons with the Sub-senior title above. In the past 5 years, the director of the department has been responsible for the organization and management of phase I clinical trials for 254 innovative new drugs. Among them, 95 new drugs belong to Category 1 (including more than 40 new drugs for liver disease, more than 20 new anti-tumor drugs, more than 10 new diabetes drugs, 5 new gynecology drugs, and more than 20 drugs targeting other systems), and more than 50 drugs have passed review by the Center for Food and Drug Inspection of National Medical Products Administration. The department has been involved in more than 40 phase II and phase III clinical trials, 3 of which have been approved for production.

The phase I clinical trial department has a well-organized, well-established, and standardized drug clinical trial management system. For example, to protect the rights and safety of subjects in clinical trials of drugs and ensure that the clinical trial results are scientific, authentic and credible, the hospital has established a sound organizational structure composed of a leading group for clinical research institutions, an ethics committee, a clinical trial management agency and its office, and a leading group for emergency organizations, to lead and organize the construction and development of the laboratory and its clinical research on drugs. The department has developed various rules & regulations and standard operating procedures for management of clinical trials of drugs; according to GCP requirements, the department designates experts with rich experience of clinical trials of drugs in various specialties and professionals who have received GCP training to revise (i.e., improve by addition and deletion of information) the existing clinical trial management system; based on the characteristics of clinical trial institutions, phase I clinical trial laboratories and specialties, the department develops precautions against damages and emergencies in subjects during medical treatment, a clinical trial management system and standard operating procedures to provide an institutional basis for the smooth progress of clinical trials in accordance with the GCP principles.

The phase I clinical trial department has an area of about 1500 square meters, 62 beds, a subject reception room, an intensive care unit for rescue, and complete rescue equipment (including the defibrillator, ventilator, ambulatory ECG monitor, and electronic sphygmomanometer). In the department, drugs are stored in dedicated cabinets managed by designated personnel. All wards are equipped with synchronized LED clocks and TV monitoring equipment, and all refrigerators are equipped with a temperature monitoring system. The department strictly follows the standards issued by the National Medical Products Administration (NMPA) and the U.S. Food and Drug Administration (FDA), and it can undertake clinical trials of drugs approved by the NMPA and FDA, including phase I clinical trials, clinical pharmacokinetic bridging studies, clinical trials to assess the mass balance of innovative drugs, glucose clamp studies, and human bioequivalence studies.

Annex 3 Clinical Laboratory Test Items

Blood biochemistry: Creatine kinase (CK), lactate dehydrogenase, creatine kinase isoenzyme, α-hydroxybutyrate dehydrogenase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total protein, albumin, globulin, albumin/globulin ratio (A/G ratio), total bilirubin, direct bilirubin, indirect bilirubin, potassium, sodium, chloride, calcium, phosphorus, cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein (VLDL), urea, creatinine, uric acid, glomerular filtration rate, fasting blood glucose, lipase **C-reactive protein**	**Hematology:** White blood cell (WBC) count, percentage of neutrophils, percentage of lymphocytes, percentage of monocytes, percentage of eosinophils, percentage of basophils, neutrophil (NEUT) count, lymphocyte (LY) count, monocyte (MONO) count, eosinophil (Eos) count, basophil count, red blood cell (RBC) count, hemoglobin (Hgb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width, platelets (PLTs), plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width
Coagulation: Prothrombin time (PT), reference interval of prothrombin time, international normalized ratio (INR), activated partial thromboplastin time (APTT), reference interval of the activated partial thromboplastin time, thrombin time (TT), fibrinogen (FIB), D-dimer	**Urinalysis:** Occult blood, leukocyte esterase (LE), protein, bilirubin, ketone, urobilinogen, nitrite, glucose, pH, specific gravity, turbidity, color, erythrocytes, leukocytes, epithelial cells, bacteria, mucus filaments, germs like yeasts, hyaline casts, granular casts, calcium oxalate crystals, uric acid crystals
Blood test for infectious diseases: Treponema pallidum-specific antibody, human immunodeficiency virus antibody, HCV immunoglobulin G (IgG) antibody, hepatitis B surface antigen, hepatitis C virus core antigen	**Serum pregnancy test** (for women of childbearing age only)

For each test in the above table, only the following items need to be included in the eCRF:

1) Blood biochemical indicators: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), creatine kinase (CK), lipase, serum creatinine (Scr), urea, C-reactive protein (CRP).
2) Hematological indicators: White blood cell (WBC) count, lymphocyte (LY) count and percentage, monocyte (MONO) count and percentage, neutrophil (NEUT) count and percentage, eosinophil (Eos) count and percentage, red blood cell (RBC) count, hemoglobin (Hgb), platelets (PLTs).
3) Coagulation indicators: Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB).
4) Urinalysis indicators: Urine protein (PRO), urine glucose, erythrocytes.
5) Serum pregnancy test (for women of childbearing age only)