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Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Yang S, Li Y, Dai L, et al. Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001) against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials. *Lancet Infect Dis* 2021; published online March 24. https://doi.org/10.1016/S1473-3099(21)00127-4.

Appendix 2

Content

1. Participants withdrew the trials

Reasons	Participants in group and quit time
In phase 1	
Quit voluntarily	2 in placebo group quit before second and third doses 2 in 25 µg group quit before second dose 3 in 25 µg group quit before third dose 1 in 50 µg group quit before third dose
Serious adverse event due to rhabdomyolysis	1 in 50 ug group quit after first dose
In phase 2	
Leaving the research center	2 in placebo, 3-dose group quit before second dose 1 in 25 µg, 3-dose group quit before second dose
Quit voluntarily	2 in placebo, 3-dose group quit before third dose 1 in 25 µg, 3-dose group quit before third dose 1 in 50 µg, 3-dose group quit before third dose
Pregnant	1 in 50 µg, 3-dose group quit after first dose

2. Adverse events and adverse reactions after each dose in phase 1 trial

	Placebo, 3-dose	25 μg, 3-dose	50 μg, 3-dose	p value
Overall adverse events within 30 days				
1st dose	4/10 (40.0%)	6/20 (30.0%)	14/20 (70.0%)	0.0383
2nd dose	4/9 (44.4%)	10/18 (55.6%)	10/19 (52.6%)	0.9283
3rd dose	3/8 (37.5%)	8/15 (53.3%)	11/18 (61.1%)	0.5549
Solicited adverse reactions within 7 days				
1st dose	1/10 (10.0%)	4/20 (20.0%)	9/20 (45.0%)	0.1016
2nd dose	2/9 (22.2%)	5/18 (27.8%)	4/19 (21.1%)	0.9040
3rd dose	2/8 (25.0%)	7/15 (46.7%)	9/18 (50.0%)	0.5563
Solicited systemic adverse reactions				
1st dose	0	2/20 (10.0%)	3/20 (15.0%)	0.6144
2nd dose	1/9 (11.1%)	2/18 (11.1%)	2/19 (10.5%)	1.0000
3rd dose	0	0	1/18 (5.6%)	1.0000
Solicited local adverse reactions				
1st dose	1/10 (10.0%)	2/20 (10.0%)	7/20 (35.0%)	0.1235
2nd dose	1/9 (11.1%)	4/18 (22.2%)	3/19 (15.8%)	0.7818
3rd dose	2/8 (25.0%)	7/15 (46.7%)	8/18 (44.4%)	0.6001
Unsolicited adverse reactions				
1st dose	3/10 (30.0%)	4/20 (20.0%)	4/20 (20.0%)	0.8190
2nd dose	4/9 (44.4%)	3/18 (16.7%)	3/19 (15.8%)	0.2398
3rd dose	1/8 (12.5%)	2/15 (13.3%)	4/18 (22.2%)	0.8679

Data are absolute no./total no. (%). P values are calculated with Fisher's exact test.

3. Adverse events and adverse reactions after each dose in phase 2 trial

	Placebo, 2-dose	25 μg, 2-dose	50 μg, 2-dose	Placebo, 3-dose	25 μg, 3-dose	50 μg, 3-dose	p value
Overall adverse events within 30 days							
1st dose	26/150 (17.3%)	25/150 (16.7%)	28/150 (18.7%)	28/150 (18.7%)	32/150 (21.3%)	27/150 (18.0%)	0.9349
2nd dose	14/150 (9.3%)	25/150 (16.7%)	30/150 (20.0%)	20/148 (13.5%)	33/150 (22.0%)	34/149 (22.8%)	0.0134
3rd dose	/	/	/	11/148 (7.4%)	40/148 (27.0%)	35/149 (23.5%)	<0.0001
Solicited adverse reactions within 7 days							
1st dose	11/150 (7.3%)	11/150 (7.3%)	13/150 (8.7%)	14/150 (9.3%)	16/150 (10.7%)	12/150 (8.0%)	0.9007
2nd dose	6/150 (4.0%)	18/150 (12.0%)	20/150 (13.3%)	7/148 (4.7%)	23/150 (15.3%)	21/149 (14.1%)	0.0017
3rd dose	/	/	/	1/148 (0.7%)	33/148 (22.3%)	25/149 (16.8%)	<0.0001
Solicited systemic adverse reactions							
1st dose	6/150 (4.0%)	10/150 (6.7%)	9/150 (6.0%)	12/150 (8.0%)	9/150 (6.0%)	7/150 (4.7%)	0.7390
2nd dose	2/150 (1.3%)	7/150 (4.7%)	7/150 (4.7%)	5/148 (3.4%)	9/150 (6.0%)	7/149 (4.7%)	0.4352
3rd dose	/	/	/	1/148 (0.7%)	1/148 (0.7%)	2/149 (1.3%)	0.7810
Solicited local adverse reactions							
1st dose	5/150 (3.3%)	3/150 (2.0%)	4 /150 (2.7%)	4/150 (2.7%)	8/150 (5.3%)	6/150 (4.0%)	0.6523
2nd dose	4/150 (2.7%)	14/150 (9.3%)	16/150 (10.7%)	3/148 (2.0%)	15/150 (10.0%)	16/149 (10.7%)	0.0033
3rd dose	/	/	/	0	32/148 (21.6%)	24/149 (16.1%)	<0.0001
Unsolicited adverse reactions							
1st dose	7/150 (4.7%)	3/150 (2.0%)	3/150 (2.0%)	4/150 (2.7%)	5/150 (3.3%)	1/150 (0.7%)	0.3495
2nd dose	2/150 (1.3%)	2/150 (1.3%)	3/150 (2.0%)	6/148 (4.1%)	5/150 (3.3%)	3/149 (2.0%)	0.5459
3rd dose	/	/	/	5/148 (3.4%)	0	5/149 (3.4%)	0.0782

Data are absolute no./total no. (%). P values are calculated with Fisher's exact test

4. Grades of adverse events and adverse reactions in phase 1 trial

	Placebo, 3-dose (n=10)	25 μg, 3-dose (n=20)	50 μg, 3-dose (n=20)	p value
Solicited adverse events within 7 days				
Any	3 (30%)	12 (60%)	14 (70%)	0.1286
Grade 1	3 (30%)	12 (60%)	14 (70%)	0.1286
Grade 2	1 (10%)	2 (10%)	5 (25%)	0.5310
≥Grade 3	0	0	1 (5%)	1.0000
Solicited systemic adverse reactions				
Any	1 (10%)	4 (20%)	5 (25%)	0.7423
Grade 1	1 (10%)	4 (20%)	5 (25%)	0.7423
Grade 2	0	1 (5%)	1 (5%)	1.0000
≥Grade 3	0	0	0	1.0000
Solicited local adverse reactions				
Any	2 (20%)	9 (45%)	12 (60%)	0.1298
Grade 1	2 (20%)	9 (45%)	12 (60%)	0.1298
Grade 2	1 (10%)	1 (5%)	4 (20%)	0.4142
≥Grade 3	0	0	1 (5%)	1.0000

Data are no. (%). P values are calculated with Fisher's exact test.

5. Grades of adverse events and adverse reactions in phase 2 trial

	Placebo, 2-dose (n=150)	25 μg, 2-dose (n=150)	50 μg, 2-dose (n=150)	Placebo, 3-dose (n=150)	25 μg, 3-dose (n=150)	50 μg, 3-dose (n=150)	p value
Solicited adverse events within 7 days							
Any	16 (10.7%)	27 (18.0%)	32 (21.3%)	19 (12.7%)	55 (36.7%)	42 (28.0%)	<0.0001
Grade 1	15 (10.0%)	24 (16.0%)	27 (18.0%)	19 (12.7%)	51 (34.0%)	41 (27.3%)	<0.0001
Grade 2	3 (2.0%)	10 (6.7%)	11 (7.3%)	3 (2.0%)	18 (12.0%)	18 (12.0%)	0.0003
≥Grade 3	0	2 (1.3%)	1 (0.7%)	1 (0.7%)	2 (1.3%)	5 (3.3%)	0.1460
Solicited systemic adverse reactions							
Any	8 (5.3%)	15 (10.0%)	16 (10.7%)	16 (10.7%)	15 (10.0%)	13 (8.7%)	0.5890
Grade 1	6 (4.0%)	12 (8.0%)	14 (9.3%)	14 (9.3%)	15 (10.0%)	13 (8.7%)	0.4521
Grade 2	2 (1.3%)	3 (2.0%)	2 (1.3%)	2 (1.3%)	1 (0.7%)	0	0.6637
≥Grade 3	0	2 (1.3%)	0	1 (0.7%)	0	0	0.2189
Solicited local adverse reactions							
Any	9 (6.0%)	17 (11.3%)	19 (12.7%)	6 (4.0%)	45 (30.0%)	35 (23.3%)	<0.0001
Grade 1	9 (6.0%)	15 (10.0%)	15 (10.0%)	6 (4.0%)	40 (26.7%)	32 (21.3%)	<0.0001
Grade 2	0	6 (4.0%)	9 (6.0%)	0	15 (10.0%)	16 (10.7%)	<0.0001
≥Grade 3	0	0	1 (0.7%)	0	2 (1.3%)	5 (3.3%)	0.0121

Data are no. (%). P values are calculated with Fisher's exact test.

6. Specific antibody responses to RBD, neutralizing antibodies to live virus at baseline

	Cohort in phase 1			Cohort in phase 2					
	Placebo, 3-dose (n=10)	25 μg, 3-dose (n=20)	50 μg, 3-dose (n=20)	Placebo, 2-dose (n=150)	25 μg, 2-dose (n=150)	50 μg, 2-dose (n=150)	Placebo, 3-dose (n=150)	25 μg, 3-dose (n=150)	50 μg, 3-dose (n=150)
Baseline									
ELISA antibodies to RBD									
≥detection limit (1:11)	0	0	0	3, 2.0%	3, 2.0%	3, 2.0%	6, 4.0%	5, 3.3%	5, 3.3%
GMT (95% CI)	5.5 (-)	5.5 (-)	5.5 (-)	5.7 (5.5-5.9)	5.7 (5.5-5.9)	5.7 (5.5-5.9)	5.9 (5.6-6.3)	5.7 (5.5-6.0)	5.8 (5.5-6.0)
Neutralizing antibodies to live SARS-CoV-2									
≥ detection limit (1:4)	0	0	0	0	0	0	0	0	0
GMT (95% CI)	2.0 (-)	2.0 (-)	2.0 (-)	2.0 (-)	2.0 (-)	2.0 (-)	2.0 (-)	2.0 (-)	2.0 (-)

Data are n, % or geometric mean titer (95% CI). The first dilution of sera is 1:11 for ELISA and 1:4 for neutralizing assay, ≥detection limit means positive. The undetectable values were assigned a value of ½ LOD (limit of detection).

7. GMT and seroconversion of specific antibody responses to RBD at various timepoints in phase 1

	Placebo, 3-dose	**25 µg, 3-dose**	**50 µg, 3-dose**	**p value**
At day 14				
FAS	n=10	n=20	n=20	
GMT (95% CI)	5.5 (-)	10.5 (6.7-16.5)	9.0 (6.3-12.9)	0.1082
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	7, 35.0% (15.4-59.2%)	7, 35.0% (15.4-59.2%)	0.0843
PPS	n=10	n=20	n=20	
GMT (95% CI)	5.5 (-)	10.5 (6.7-16.5)	9.0 (6.3-12.9)	0.1082
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	7, 35.0% (15.4-59.2%)	7, 35.0% (15.4-59.2%)	0.0843
At day 30				
FAS	n=10	n=20	n=20	
GMT (95% CI)	5.5 (-)	24.1 (12.4-46.7)	36.9 (20.3-67.3)	0.0008
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	13, 65.0% (40.8-84.6%)	15, 75.0% (50.9-91.3%)	0.0002
PPS	n=9	n=18	n=19	
GMT (95% CI)	5.5 (-)	23.1 (11.2-47.9)	40.8 (22.5-74.0)	0.0009
Seroconversion, rate (95% CI)	0, 0 (0-33.6%)	11, 61.1% (35.8-82.7%)	15, 79.0% (54.4-94.0%)	0.0002
At day 37				
FAS	n=10	n=20	n=20	
GMT (95% CI)	5.5 (-)	65.1 (37.6-112.9)	110.2 (63.5-191.2)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	19, 95.0% (75.1-99.9%)	19, 95.0% (75.1-99.9%)	<0.0001
PPS	n=9	n=18	n=19	
GMT (95% CI)	5.5 (-)	69.8 (38.6-126.4)	129.0 (80.9-205.8)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-33.6%)	17, 94.4% (72.7-99.9%)	19, 100% (82.4-100%)	<0.0001
At day 60				

FAS	n=10	n=20	n=20	
GMT (95% CI)	5.5 (-)	462.3 (200.7-1064.9)	593.7 (289.9-1216.0)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	19, 95.0% (75.1-99.9%)	19, 95.0% (75.1-99.9%)	<0.0001
PPS	n=8	n=15	n=18	
GMT (95% CI)	5.5 (-)	1077.0 (663.7-1747.5)	825.5 (486.9-1399.4)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-36.9%)	15, 100% (78.2-100%)	18, 100% (81.5-100%)	<0.0001
At day 67				
FAS	n=10	n=20	n=20	
GMT (95% CI)	5.5 (-)	740.0 (275.3-1988.9)	1557.2 (671.3-3612.6)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	19, 95.0% (75.1-99.9%)	19, 95.0% (75.1-99.9%)	<0.0001
PPS	n=8	n=15	n=18	
GMT (95% CI)	5.5 (-)	2016.2 (1081.3-3759.5)	2410.0 (1382.8-4200.0)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-36.9%)	15, 100% (78.2-100%)	18, 100% (81.5-100%)	<0.0001
At day 90				
FAS	n=10	n=20	n=20	
GMT (95% CI)	5.5 (-)	926.1 (334.9-2561.1)	1769.0 (809.2-3867.3)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	19, 95.0% (75.1-99.9%)	19, 95.0% (75.1-99.9%)	<0.0001
PPS	n=8	n=15	n=18	
GMT (95% CI)	5.5 (-)	2719.5 (1584.1-4668.8)	2776.8 (1875.5-4111.2)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-36.9%)	15, 100% (78.2-100%)	18, 100% (81.5-100%)	<0.0001

Data are geometric mean titer (95% CI) or no. of seroconversion, % (95% CI). P values of GMT are calculated with ANOVA. P values of seroconversion rates are calculated with Fisher's exact test. FAS, Full Analysis Set, including the participants who followed the principle of Intent to Treat, received at least one dose, completed pre-blood collection and had effective immune evaluation indicators; PPS, Per Protocol Set, including the participants who were more compliant with the protocol, met the inclusion/exclusion criteria and received vaccinations as required.

8. GMT and seroconversion of neutralizing antibodies to live SARS-CoV-2 at various timepoints in phase 1

	Placebo, 3-dose	**25 μg, 3-dose**	**50 μg, 3-dose**	**p value**
At day 30				
FAS	n=10	n=20	n=20	
GMT (95% CI)	2.0 (-)	2.0 (-)	2.1 (1.9-2.4)	0.2194
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	0, 0 (0-16.8%)	2, 10.0% (1.2-31.7%)	0.3469
PPS	n=9	n=18	n=19	
GMT (95% CI)	2.0 (-)	2.0 (-)	2.2 (1.9-2.4)	0.2380
Seroconversion, rate (95% CI)	0, 0 (0-33.6%)	0, 0 (0-18.5%)	2, 11% (1.3-33.1%)	0.6696
At day 60				
FAS	n=10	n=20	n=20	
GMT (95% CI)	2.1 (1.8-2.5)	8.6 (4.8-15.3)	9.6 (5.5-16.6)	0.0019
Seroconversion, rate (95% CI)	1, 10.0% (0.3-44.5%)	14, 70.0% (45.7-88.1%)	17, 85.0% (62.1-96.8%)	0.0003
PPS	n=8	n=15	n=18	
GMT (95% CI)	2.2 (1.8-2.7)	14.0 (8.0-24.6)	11.4 (6.6-19.8)	0.0002
Seroconversion, rate (95% CI)	1, 12.5% (0.3-52.7%)	14, 93.3% (68.1-99.8%)	17, 94.4% (72.7-99.9%)	<0.0001
At day 67				
FAS	n=10	n=20	n=20	
GMT (95% CI)	2.0 (-)	36.0 (14.2-91.4)	78.4 (35.4-173.5)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-30.9%)	15, 75.0% (50.9-91.3%)	18, 90.0% (68.3-98.8%)	<0.0001
PPS	n=8	n=15	n=18	
GMT (95% CI)	2.0 (-)	94.5 (49.3-181.3)	117.8 (64.6-214.9)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-36.9%)	15, 100% (78.2-100%)	18, 100% (81.5-100%)	<0.0001

Data are geometric mean titer (95% CI) or no. of seroconversion, % (95% CI). P values of GMT are calculated with ANOVA. P values of seroconversion rates are calculated with Fisher's exact test.

9. GMT and seroconversion of specific antibody responses to RBD at various timepoints in phase 2

	Placebo, 2-dose	25 μg, 2-dose	50 μg, 2-dose	Placebo, 3-dose	25 μg, 3-dose	50 μg, 3-dose	p value
At day 14							
FAS	n=150	n=150	n=150	n=150	n=150	n=150	
GMT (95%CI)	5.7 (5.5-5.9)	7.1 (6.4-7.9)	7.7 (6.8-8.6)	5.9 (5.6-6.2)	7.4 (6.6-8.2)	6.7 (6.1-7.4)	<0.0001
PPS	n=150	n=150	n=150	n=148	n=150	n=149	
GMT (95%CI)	5.7 (5.5-5.9)	7.1 (6.4-7.8)	7.7 (6.8-8.6)	5.9 (5.6-6.2)	7.4 (6.6-8.2)	6.7 (6.1-7.4)	<0.0001
FAS	n=147	n=147	n=147	n=144	n=145	n=145	
Seroconversion, rate (95%CI)	1, 0.7% (0-3.7%)	23, 15.7% (10.2-22.6%)	27, 18.3% (12.5-25.6%)	0, 0 (0-2.5%)	23, 15.9% (10.3-22.8%)	14, 9.7% (5.4-15.7%)	<0.0001
PPS	n=147	n=147	n=147	n=142	n=145	n=144	
Seroconversion, rate (95%CI)	1, 0.7% (0-3.7%)	23, 15.7% (10.2-22.6%)	27, 18.3% (12.5-25.6%)	0, 0 (0-2.6%)	23, 15.9% (10.3-22.8%)	14, 9.7% (5.4-15.8%)	<0.0001
At day 30							
FAS	n=150	n=150	n=150	n=150	n=150	n=150	
GMT (95%CI)	5.7 (5.4-6.0)	19.4 (16.0-23.6)	22.6 (18.5-27.6)	5.8 (5.6-6.2)	24.4 (19.6-30.2)	22.2 (18.0-27.2)	<0.0001
PPS	n=150	n=150	n=150	n=147	n=150	n=148	
GMT (95%CI)	5.7 (5.4-6.0)	19.4 (16.0-23.6)	22.6 (18.5-27.6)	5.9 (5.6-6.2)	24.4 (19.6-30.2)	22.2 (18.1-27.4)	<0.0001
FAS	n=147	n=147	n=147	n=144	n=145	n=145	
Seroconversion, rate (95%CI)	1, 0.7% (0-3.7%)	87, 59.2% (50.8-67.2%)	96, 65.3% (57.0-73.0%)	0, 0 (0-2.5%)	92, 63.5% (55.1-71.3%)	90, 62.1% (53.6-70.0%)	<0.0001
PPS	n=147	n=147	n=147	n=141	n=145	n=143	
Seroconversion, rate (95%CI)	1, 0.7% (0-3.7%)	87, 59.2% (50.8-67.2%)	96, 65.3% (57.0-73.0%)	0, 0 (0-2.6%)	92, 63.5% (55.1-71.3%)	89, 62.2% (53.8-70.2%)	<0.0001
At day 44							
FAS	n=150	n=150	n=150	n=150	n=150	n=150	
GMT (95%CI)	6.0 (5.4-6.6)	439.8 (323.8-597.5)	338.0 (252.4-452.8)	5.8 (5.6-6.2)	483.9 (365.4-640.9)	262.9 (192.9-358.3)	<0.0001
PPS	n=150	n=150	n=150	n=147	n=150	n=148	

GMT (95%CI)	6.0 (5.4-6.6)	439.8 (323.8-597.5)	338.0 (252.4-452.8)	5.9 (5.6-6.2)	483.9 (365.4-640.9)	265.4 (195.1-361.1)	<0.0001
FAS	n=147	n=147	n=147	n=144	n=145	n=145	
Seroconversion, rate (95%CI)	2, 1.4% (0.2-4.8%)	136, 92.5% (87.0-96.2%)	137, 93.2% (87.9-96.7%)	0, 0 (0-2.5%)	136, 93.8% (88.5-97.1%)	128, 88.3% (81.9-93.0%)	<0.0001
PPS	n=147	n=147	n=147	n=141	n=145	n=143	
Seroconversion, rate (95%CI)	2, 1.4% (0.2-4.8%)	136, 92.5% (87.0-96.2%)	137, 93.2% (87.9-96.7%)	0, 0 (0-2.6%)	136, 93.8% (88.5-97.1%)	127, 88.8% (82.5-93.5%)	<0.0001
At day 60							
FAS	n=150	n=150	n=150	n=150	n=150	n=150	
GMT (95%CI)	6.0 (5.4-6.6)	419.5 (325.8-540.1)	344.8 (271.0-438.7)	5.8 (5.5-6.1)	475.0 (372.7-605.4)	299.1 (230.7-387.7)	<0.0001
PPS	n=150	n=150	n=150	n=147	n=149	n=148	
GMT (95%CI)	6.0 (5.4-6.6)	419.5 (325.8-540.1)	344.8 (271.0-438.7)	5.8 (5.5-6.2)	467.3 (366.8-595.3)	302.5 (234.2-390.6)	<0.0001
FAS	n=147	n=147	n=147	n=144	n=145	n=145	
Seroconversion, rate (95%CI)	2, 1.4% (0.2-4.8%)	140, 95.2% (90.4-98.1%)	142, 96.6% (92.2-98.9%)	0, 0 (0-2.5%)	139, 95.9% (91.2-98.5%)	135, 93.0% (87.7-96.6%)	<0.0001
PPS	n=147	n=147	n=147	n=141	n=145	n=143	
Seroconversion, rate (95%CI)	2, 1.4% (0.2-4.8%)	140, 95.2% (90.4-98.1%)	142, 96.6% (92.2-98.9%)	0, 0 (0-2.6%)	139, 95.9% (91.2-98.5%)	134, 93.7% (88.4-97.1%)	<0.0001
At day 74							
FAS				n=150	n=150	n=150	
GMT (95%CI)				5.8 (5.6-6.1)	1745.7 (1403.7-2171.0)	1107.4 (851.4-1440.3)	<0.0001
PPS				n=146	n=148	n=148	
GMT (95%CI)				5.9 (5.6-6.2)	1782.3 (1440.2-2205.7)	1140.0 (882.2-1473.2)	<0.0001
FAS				n=144	n=145	n=145	
Seroconversion, rate (95%CI)				0, 0 (0-2.5%)	144, 99.3% (96.2-100%)	140, 96.5% (92.1-98.9%)	<0.0001
PPS				n=140	n=144	n=143	

Seroconversion, rate (95%CI)				0, 0 (0-2.6%)	143, 99.3% (96.2-100%)	139, 97.2% (93.0-99.2%)	<0.0001

Data are geometric mean titer (95% CI) or no. of seroconversion, % (95% CI), The no. of seroconversion in PPS and FAS excludes those with baseline in Appendix 4. P values of GMT are calculated with ANOVA. P values of seroconversion rates are calculated with Chi-square test.

10. GMT and seroconversion of neutralizing antibodies to live SARS-CoV-2 at various timepoints in phase 2

	Placebo, 2-dose	25 μg, 2-dose	50 μg, 2-dose	Placebo, 3-dose	25 μg, 3-dose	50 μg, 3-dose	p value
At day 44							
FAS	n=150	n=150	n=150	n=150	n=150	n=150	
GMT (95% CI)	2.0 (-)	17.7 (13.6-23.1)	14.1 (10.8-18.3)	2.1 (2.0-2.1)	19.2 (15.0-24.6)	12.8 (10.1-16.3)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-2.4%)	114, 76.0% (68.4-82.6%)	108, 72.0% (64.1-79.0%)	2, 1.3% (0.2-4.7%)	124, 82.7% (75.6-88.4%)	109, 72.7% (64.8-79.6%)	<0.0001
PPS	n=150	n=150	n=150	n=147	n=149	n=148	
GMT (95% CI)	2.0 (-)	17.7 (13.6-23.1)	14.1 (10.8-18.3)	2.1 (2.0-2.1)	19.5 (15.2-25.0)	12.6 (10.0-16.0)	<0.0001
Seroconversion, rate (95% CI)	0, 0 (0-2.4%)	114, 76.0% (68.4-82.6%)	108, 72.0% (64.1-79.0%)	2, 1.4% (0.2-4.8%)	124, 83.2% (76.2-88.8%)	108, 73.0% (65.1-79.9%)	<0.0001
At day 74							
FAS				n=150	n=150	n=150	
GMT (95% CI)				2.0 (-)	97.3 (76.9-123.0)	68.4 (52.4-89.2)	<0.0001
Seroconversion, rate (95% CI)				0, 0 (0-2.4%)	143, 95.3% (90.6-98.1%)	139, 92.7% (87.3-96.3%)	<0.0001
PPS				n=146	n=148	n=148	
GMT (95% CI)				2.0 (-)	102.5 (81.8-128.5)	69.1 (53.0-90.0)	<0.0001
Seroconversion, rate (95% CI)				0, 0 (0-2.5%)	143, 96.6% (92.3-98.9%)	138, 93.2% (87.9-96.7%)	<0.0001

Data are geometric mean titer (95% CI) or no. of seroconversion, % (95% CI). P values of GMT are calculated with ANOVA. P values of seroconversion rates are calculated with Chi-square test.

11. The list of serious adverse events in phase 1 and 2 trails

No. of participant	Gruop	Gender	Age (year)	Vaccination date	System organ classification	Preference terms	Serious adverse events description	Start date	End date	Duration (day)	Serious adverse event type	Correlation to vaccine	Correlation analysis	Consequence	Withdraw
44*	50 ug, 3-dose	M	27.7	7/3/2020	Various musculoskeletal and connective tissue diseases	Rhabdomyolysis	Blood biochemical test results: Creatine kinase, 33002 U/L; Aspertate aminotransferase, 301 U/L; Alanine aminotransferase, 94 U/L; Lactate dehydrogenase, 888 U/L; α- Hydroxybutyric dehydrogenase, 376 U/L; CreatineKinase-MB, 5.8 ng/mL (reference interval ⩽ 5.0 ng/mL); Cardiac troponin I and brain natriuretic peptide were normal. The abnormal value of CKMB had no clinical significance for doctors to judge the state of illness.	8/2/2020	8/11/2020	10	Life-threatening	likely unrelated	Based on subject's disease presentation and clinical course, subject suffered from relatively mild " cough " which was not treated with special care and did not meet SAE criteria. The subject was hospitalized due to " rhabdomyolysis ", met SAE criteria, considered that the subject had abnormal liver function 30 days after the 1st dose of vaccine, so the decision was likely unrelated to the study vaccination .	Cured	Yes
045	50 ug, 2-dose	F	56.3	7/12/2020; 8/14/2020	Various types of injuries, poisoning and operational complications	Bone contusion	The posterior horn of medial meniscus of right joint was degenerated, the knee movement was limited.	8/2/2020	8/14/2020	13	Prolonged hospitalization or hospitalization	Unrelated	Based on the subject's disease presentation, the patient was hospitalized due to " right distal radius fracture " caused by inadvertent fall, met SAE criteria and	Remission	No
						Radius fracture	The right wrist was tender slightly.								

													could be judged as other significant cause, so consider this SAE as " right distal radius fracture " caused, not related to study vaccination definitely.The subject was hospitalized due to " right patella contusion " caused by an inadvertent fall and also met SAE criteria and was additionally reported as a separate SAE in summary.		
158	25 ug, 3-dose	M	36.1	7/13/2020; 8/14/2020; 9/15/2020	Infection disease Kidney and uropoietic system diseases	Ureteritis Renal lithiasis Ureteral calculi	Right waist and abdomen were in persistent pain, nausea, fever, no vomiting, no chills.	10/9/2020	10/11/2020	3	Prolonged hospitalization or hospitalization	Unrelated	Based on the subject's disease presentation, the subject was hospitalized due to " right ureteral stone and infection ", met the criteria for SAE, considered other significant causes, so the initial decision that this SAE was definitely unrelated to the study vaccination.	Cured	No
178	25 ug, 2-dose	F	49.9	7/14/2020; 8/15/2020; 9/16/2020	Various types of injuries, poisoning and	Ligament injury	Bilateral knee joints were red, swelling and painful, effusion was in intra-knee	9/12/2020	9/17/2020	6	Prolonged hospitalization or	Unrelated	Based on the subject's disease presentation, the subject was hospitalized due to "	Remission	No

					operational complications						hospitalization		double knee arthritis and anterior cruciate ligament injury of both knees", met the criteria for SAE, considered other significant cause, so the initial decision that this SAE was definitely unrelated to the study vaccination.		
					Various musculoskeletal and connective tissue diseases	Arthritis	Effusion was in bilateral intra-knees.								
256	Placebo, 3-dose	F	26.3	7/14/2020; 8/16/2020; 9/15/2020	Infection disease	Bronchitis	The foam sputum was white. It is difficult to cough, and with a slight itches in the pharynx.	8/31/2020	9/4/2020	5	Prolonged hospitalization or hospitalization	Possibly unrelated	According to the subject's disease manifestations and clinical experience, the subject's first time of cough (21-jul) was within 7 days of the first dose of the test vaccine, and there was no obvious trigger, so it was judged that this AE was possibly related to vaccination with the test vaccine.On 31 Aug was hospitalized due to " cough challenging ", met SAE criteria, transbronchial fibroscopy combined with clinical manifestations, definite diagnosis was " acute bronchitis	Remission	No

													", consider acute bronchitis caused by a large possibility of cough, so judged this SAE may not be related to study vaccination.		
266	50 ug, 3-dose	F	41.9	7/14/2020	Pregnancy, puerperium and perinatal period	Missed abortion	Abortion	9/29/2020	10/3/2020	5	Prolonged hospitalization or hospitalization	Possibly unrelated	Per subject disease presentation and clinical course, subject suffered from " post cholecystectomy, urinary protein investigations: cardiovascular disease? Urinary disease? " no significant symptoms and did not meet SAE criteria.The subject was hospitalized due to " missed abortion " considering all the causes of " visual clearing the uterus and intrauterine adhesions ", the subject was hospitalized due to " missed abortion ", met the SAE criteria, was judged to be probably not related to the study vaccination because	Cured	No

													the subject was considered to have a positive urine pregnancy test 31 days after the first dose of the test vaccine and had a previous history of adverse abortion.		
502	Placebo, 3-dose	F	42.7	7/15/2020; 8/16/2020; 9/15/2020	Infection disease	Gastroenteritis	Paroxysmal colic was around the umbilical cord, diarrhea 3-4 times per day with watery stool, no vomiting.	9/2/2020	9/8/2020	7	Prolonged hospitalization or hospitalization	Possibly unrelated	According to the disease manifestations and clinical course of the subject, the subject suffered from " right kidney stones, pelvic effusion " which was relatively mild, and none of them were given special treatment, none of them met SAE criteria.The subject was hospitalized due to " acute gastroenteritis ", met SAE criteria, considering the subject developed abdominal pain, diarrhea 15 days after the 2nd dose of vaccine, so the decision was not related to study vaccination may not	Remission	No
					Systemic diseases and various responses at the site of drug administration	Thoracodynia	Paroxysmal pain was in front chest, with back pain,and pain was aggravated when lying flat.								

													be related.Subject was hospitalized due to " chest pain investigation: pleuritis? Angina? Other? " which met SAE criteria considering subject had chest pain 15 days after 2nd dose of vaccine and chest CT		
600	50 ug, 3-dose	M	56	7/15/2020; 8/15/2020	Various types of injuries, poisoning and operational complications	Foot fracture	The left foot was swelling obviously and had local bruis. The lateral back of the foot was tender and the fracture was palpable.	8/24/2020	9/5/2020	13	Prolonged hospitalization or hospitalization	Unrelated	Based on the subject's disease presentation, the subject was hospitalized due to " left 5th metatarsal base fracture ", met the criteria for SAE, considered other significant cause resulted, inadvertent sprain caused " left 5th metatarsal base fracture ", so the consideration of this SAE was unrelated to the study vaccination may be considered.	Remission	No

* This participant was in phase 1, others were in phase 2. F, female; M, male.

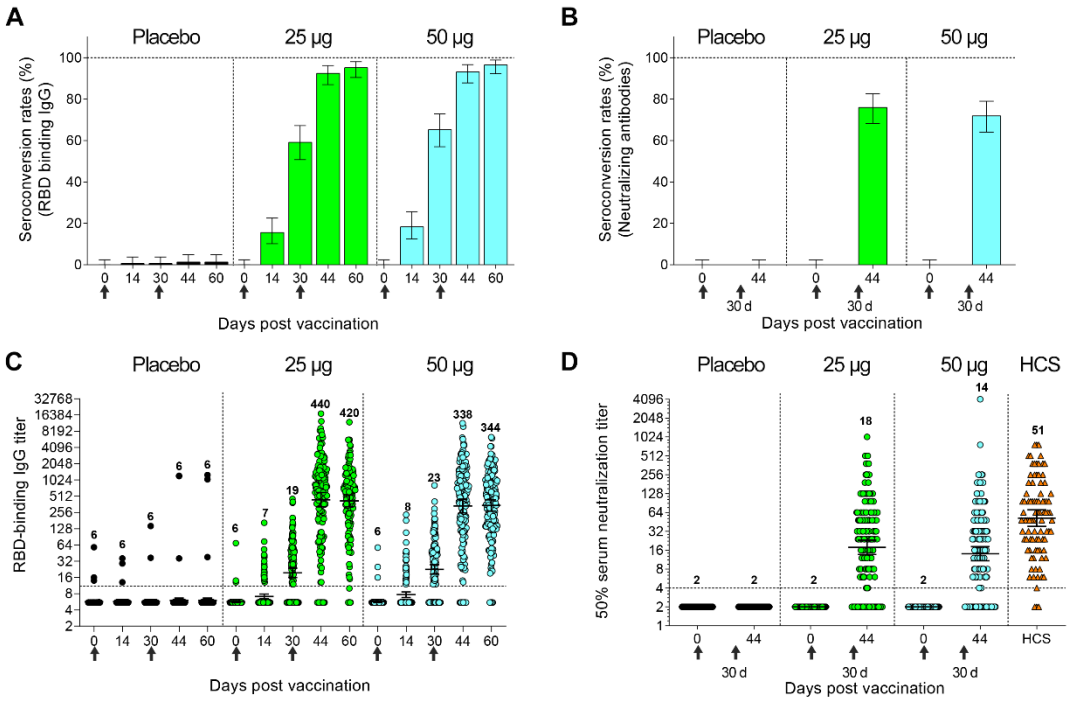
12. The information of convalescents

No.	Age (years)	Gender	Days of sera collect after symptom onset
1	51	Male	36
2	39	Male	13
3	32	Female	14
4	37	Male	5
5	23	Male	5
6	50	Male	10
7	43	Male	3
8	34	Male	5
9	36	Male	5
10	22	Female	10
11	22	Male	5
12	55	Female	6
13	40	Male	4
14	32	Male	4
15	37	Male	4
16	29	Male	6
17	27	Female	4
18	17	Female	4
19	54	Female	5
20	78	Female	5
21	43	Male	3
22	56	Female	3
23	79	Female	5
24	41	Female	10
25	15	Male	4
26	45	Male	4
27	37	Female	5
28	22	Male	unknown
29	40	Male	10
30	40	Male	11
31	44	Male	8
32	45	Female	8
33	46	Male	15
34	33	Female	16
35	62	Male	9
36	32	Female	10
37	52	Male	14
38	32	Male	18
39	36	Male	16
40	67	Female	24
41	20	Male	42
42	40	Female	67
43	36	Female	64
44	23	Female	70
45	50	Male	78

46	29	Male	137
47	56	Male	37
48	30	Male	42
49	36	Female	38
50	44	Male	37
51	61	Female	37
52	27	Male	38
53	32	Male	33
54	38	Male	37
55	29	Male	40
56	48	Male	40
57	42	Male	41
58	42	Male	43
59	50	Male	43
60	35	Female	40
61	26	Male	46
62	54	Male	40
63	28	Female	41
64	47	Male	47
65	31	Male	40
66	42	Female	41
67	51	Male	47
68	44	Female	44
69	57	Male	46
70	39	Male	46
71	36	Female	46
72	36	Female	46
73	34	Female	46
74	64	Male	75
75	47	Female	48
76	30	Male	46
77	33	Female	47
78	42	Male	46
79	36	Male	47
80	45	Male	50
81	39	Male	47
82	47	Female	50
83	61	Female	47
84	59	Female	54
85	36	Female	36
86	48	Female	49
87	53	Female	46
88	50	Female	44
89	49	Male	44

No. of male	48/81		No. of female	33/81	
Mean age	40.7	Max age	79	Min age	15
Mean days of sera collect after symptom onset				32.3	

13. Figure of humoral immune responses of 2-dose in phase 2 trial



Seroconversion rates (A) and geometric mean titres (GMTs) (C) of binding antibody at different time points after vaccination are shown. Seroconversion rates (B) and GMTs (D) of neutralizing antibody at different time points after vaccination are shown. Error bars show 95% confidence intervals (CIs). The horizontal dashed line indicated the limit of detection. HCS = human convalescent serum. 1st and 2nd indicate first and second vaccination.

Supplementary Methods

Live SARS-CoV-2 virus amplication and titration

SARS-CoV-2 virus (hCoV-19/China/CAS-B001/2020, GISAID No. EPI_ISL_514256-7) was propagated on Vero E6 cells for 3 days. The virus titer was determined with cytopathic efficiency (CPE) assay. 100 μL 1.5 × 10^5/mL cells were seeded in 96-well culture plate in advance. After 18-24 h, virus was serially diluted in 10-fold and added on the cells. Cells were cultured in a 5% CO_2 incubator at 37°C and checked under a microscope for the presence of CPE after 3 days. Virus titer was calculated with the method of Karber.

Live SARS-CoV-2 neutralization assay

The microcyto pathogenic effect assay was used to determine 50% neutralization titer. In brief, each serum was taken partly and diluted with an equal volume DMEM, incubated at 56 °C for 30 min and then diluted in 2-fold serially. Two repeats were set for each serum. 50 μL serially diluted sera were mixed with 50 μL 100 $TCID_{50}$ virus per well in 96-well plates, subsequently incubated in 5% CO_2 incubators for 2 hours at 37 °C. Both virus and sera were diluted in DMEM with 5% FBS. Vero E6 cells were resuspended in DMEM with 10% FBS in density 1.2-1.6 × 10^5/mL, 100 μL cells per well were then added to the mixtures of sera/virus. The final concentrations of FBS were 7.5%. FBS was inactivated before adding to DMEM. After the plates incubating for 3 days, the CPE of each well was recorded under microscope. Titers expressed as the reciprocal of the highest dilution protecting 50% cell from virus challenge. Samples with values ≥ 4 were defined as seroconverted.

Evaluation of specific IgG antibodies binding to SARS-CoV-2 RBD

The assay was performed with the kits of IgG antibody detection of SARS-CoV-2 RBD (WANTAI BioPharm) by indirect ELISA assay. Briefly, 96-well plates were coated with purified SARS-CoV-2 RBD protein and blocked in advance.The sera were inactivated at 56 ºC for 30 min for safety consideration, diluted in 2-fold with a 1:11 dilution as a starting concentration and then applied to 96-well plates for 30 min at 37 ºC. After washing 5 times, antibody binding was revealed using anti-human IgG labeled by HRP. Subsequently, substrate solution and stop solution were added sequentially, plate absorbance was read at 450 nm and 630 nm after reaction stop. Value of cutoff was 0.16 plus average value of negative control. IgG titer was the dilution number of endpoint multiplied value of endpoint and divided by value of cutoff. Samples with values ≥ 11 were defined as seroconverted, but those positive on day 0 (Appendix 4) were excluded in the no. of seroconversion.

Enzyme-linked immunospot (ELISpot) assay

The Human interferon γ (IFN-γ), IL-2, IL-4 and IL-5 ELISpot kit (BD Biosciences) were used to evaluate T cell responses elicited by the vaccine in clinical samples collected in various timepoint. The assay was performed as the manuscript of the kit. Briefly, frozen peripheral blood mononuclear cells (PBMC) were thawed, counted and rested in RPMI 1640 culture media with 10% FBS overnight at 37°C with 5% CO_2. Meanwhile, the capture antibody was diluted and added to each well of the ELISpot plates which were kept at 4 °C overnight afterwards. On day 2, cells were counted and resuspended in culture media with 3×10^6 cells /mL. After plates washing and blocking, 100 μL cell were transferred to individual wells of 96-well plate. Add specific peptides, mixture of PMA/Ionomycin as antigen specific stimulation and positive control. Medium w/o cells as negative and blank control. Then culture 20-24 h for IFN-gamma and IL-2, 40-48 h for IL-4and IL-5 at 37°C with 5% CO2. After washing with deionized water and wash buffer, diluted biotinylated detection antibody and streptavidin-HRP sequentially were added to the plates and incubated for 2 h or 1 h at room temperature followed washing.

Subsequently, substrate solution was added to each well and spots development was monitored from 5-60 min without light. After reaction stopping by washing wells with DI water, plates were air-dried at room temperature and stored in the dark. Spots could be enumerated automatically using CTL ImmunoSpot reader and calculated.

Safety and tolerability of a Recombinant Novel Coronavirus Vaccine (CHO cells): a multi-center, double-blind, randomized, placebo-controlled parallel phase Ⅰ clinical trial in healthy volunteers aged from 18 to 59 years old

Product name：	Recombinant novel coronavirus vaccine (CHO cell)
Drug clinical trial approval	2020L00023, 2020L00024
Approval time	June 19, 2020
Registration category	Preventive biological products
Protocol number:	LKM-2020-NCV01
Version date:	August 14, 2020
Version number:	Version 3.0
Sponsor (stamped)	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.
Research time	June 2020 to September 2021

Table of contents

Sponsor approval

Protocol Number	LKM-2020-NCV01
Version date	August 14, 2020
Version number	3.0
Study title	Safety and tolerability of a Recombinant Novel Coronavirus Vaccine (CHO cells): a multi-center, double-blind, randomized, placebo-controlled parallel phase Ⅰ clinical trial in healthy volunteers aged from 18 to 59 years old
Sponsor	Company: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. Address: No.100 Fushan Road, High-tech Zone, Hefei City, Anhui Province Postcode: 230088
Sponsor manager	Name: Shilong Yang Tel：15155935085 Email：yangshilong@zhifeishengwu.com
Sponsor Contact	Name: Zaixin Zhong Tel：18130032795 Email：zhongzaixin@zhifeishengwu.com
Scheme approval	Name: Shilong Yang Signature: Date of approval: (mm / dd / yyyy)

Signature page of principal investigator

Protocol number	LKM-2020-NCV01
Version date	August 14, 2020
Version number	Version 3.0
Official title	Safety and tolerability of a Recombinant Novel Coronavirus Vaccine (CHO cells): a multi-center, double-blind, randomized, placebo-controlled parallel phase Ⅰ clinical trial in healthy volunteers aged from 18 to 59 years old

I agree:

- Be responsible for the correct guidance of the clinical study in the region.
- Ensure that the study is conducted in accordance with the trial protocol and the standard operating procedures (SOP) for clinical studies
- Ensure that the personnel involved in the study are fully aware of the product information and other research related responsibilities and obligations specified in the trial protocol.
- Ensure that no changes are made to the trial protocol without the review and written approval of the sponsor and the Ethics Committee (IRB), except for the need to eliminate the immediate harm to the subjects or to comply with the requirements of the drug regulatory agency (e.g. administrative aspects of the project).
- Be fully familiar with the correct use of the vaccine described in the trial protocol, and fully understand other information provided by the sponsor, including but not limited to the following contents: current researcher's manual or equivalent document.
- Be familiar with and will comply with *GCP*, *guidelines for quality management of vaccine clinical trials (Trial)* and all current regulatory requirements.

The duty	**Name Organization (seal) Autograph Date of signature**

Principal investigator	Hong Ren, Xian Yu	The Second Affiliated Hospital of Chongqing Medical University		yyyy / mm / dd

Signature page of principal investigator

Protocol number	LKM-2020-NCV01
Version date	August 14, 2020
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The duty	**Name Organization (seal) Autograph Date of signature**

Principal investigator	Lihong Liu	Beijing Chaoyang Hospital, Capital Medical University		yyyy / mm / dd

Abbreviation list

English abbreviations	English full name
ADE	antibody dependence enhancement
AE	Adverse Event
ALT	Alanine aminotransferase
Arthus	Arthus Reaction
AST	Aspartate aminotransaminase
BAT-SL-COVZC45	/
BMI	Body Mass Index
COPD	Chronic obstructive pulmonary diseases
CR	Creatinine
CRO	Contract Research Organization
DM	Data management
DSMB	Data Safety and Monitoring Boards
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture System
FAS	Full Analysis Set
GCP	Good Clinical Practice
GLU	Blood Glucose
HUH-7	Human hepatoma
ICF	Informed Consent Form
IFN-γ	Interferon-γ
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-2	Interleukin-2
IRB	Institutional Review Board
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
MERSR-COV	Middle East Respiratory Syndrome Coronavirus
NMPA	National Medical Products Administration
PLT	Blood Platelet
PPS	Per Protocol Set
PRO	Urine Protein
PV	Pharmacovigilance

RBC	Red Blood Cell
RBD	receptor-binding domain
RT-PCR	Reverse transcription PCR
SAE	Serious Adverse Event
SARS	Severe acute respiratory syndrome
SARS-coV-2	Severe acute respiratory syndrome-Cornona Virus Disease
SAS	Statistical analysis system
SOP	Standard Operation Procedure
SS	Safety Set
Stevens-Johnsons 综合症	Stevens-Johnsons syndrome
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
VERO E6	Monkey kidney cell
WBC	White Blood Cell
WHO	World Health Organization

Glossary of terms

Case report form	A printed, optical or electronic document that is reported to the sponsor and designed according to the requirements of the trial protocol, which is used to record all informations of each subject during the trial.
Audit	Conduct systematic and independent verification of all behaviors and related documents of clinical trials. To determine whether the implementation process of the trial and the recording, analysis and report of the test data are consistent with the requirements of the trial protocol, standard operating procedures (SOP), Good Clinical Practice (GCP) and current relevant laws and regulations.
Monitor	Supervise and review the progress and process of clinical trials to ensure that clinical trials are implemented, recorded and reported in accordance with the trial protocol, SOP, GCP and relevant laws and regulations.
Randomization	The principle of randomization in clinical trials refers to the implementation process or measures in which each subject has the equal opportunity to be assigned to the experimental group or the control group in the clinical trial, and the randomization process is not affected by the subjective wishes of the investigators and / or the subjects.
researchvaccine	Vaccines used in clinical trials include trial vaccines and placebo.
Subjects	Individuals, including healthy volunteers and patients, who voluntarily participate in a clinical trial and act as the recipient of the research vaccine or as the trial control.
Drop out	It means that the subject cannot continue to follow the trial protocol to the required last follow-up for any reason.
Investigator's brochure	The clinical and non-clinical research data of the relevant experimental vaccines in human trials.

Adverse event	All adverse medical events that occurred after subjects received the experimental vaccine, but not necessarily causally related to treatment.
Soliciting adverse events	Adverse events collected as safety endpoints in clinical studies refer to information on adverse events actively collected by investigators or subjects during a specific follow-up period after vaccination.
Non-solicitation adverse events	Other adverse events other than solicitation adverse events reported in clinical studies also include solicitation adverse events reported outside the designated solicitation time window.
Serious adverse e vent	The subject has died, life-threatening, permanent or severe disability or loss of function after receiving the experimental vaccine, the subject needs to be hospitalized or extended hospital stay, and congenital abnormalities or birth defects and other adverse medical events.
Data Safety and monitoring Board	An independent committee composed of a group of professionals with relevant professional knowledge and experience. The sponsor can establish a regular evaluation of clinical trial progress, safety data and key efficacy indicators, and recommend whether to continue, modify or stop the trial.

Research team

Protocol Number	LKM-2020-NCV01
Version date	August 14, 2020
Version number	3.0
Study title	Safety and tolerability of a Recombinant Novel Coronavirus Vaccine (CHO cells): a multi-center, double-blind, randomized, placebo-controlled parallel phase Ⅰ clinical trial in healthy volunteers aged from 18 to 59 years old
List of clinical trial related organizations	
Research Center 1 (team leader): the Second Affiliated Hospital of Chongqing Medical University Address: 76 Linjiang Road, Yuzhong District, Chongqing Postcode: 400010 Principal investigators:Ren Hong,Yu Xian Tel: 023-63693790 E-mail：22168398@qq.com Professional department: phase I clinical laboratory	
Research Center 2: Beijing Chaoyang Hospital , Capital Medical University Address: No.8, Gongti South Road, Chaoyang District, Beijing Postcode: 100020 Principal investigator:Liu Lihong Tel:010-85231788，13718936779 E-mail：hongllh@126.com	

Professional department: phase I clinical laboratory

Data and Security Oversight Board (DSMB)

Chairman:

Name: Xia Jielai

Organization: Department of health statistics, Department of preventive medicine, Air Force Medical University

Tel：029-84774804

E-mail：13571999716@163.com

Expert committee 1:

Name: Wang Xuanyi

Organization: Institutes of Biomedical Sciences Fudan University

Tel：021-54237865

E-mail：xywang@shmu.edu.cn

Expert committee 2:

Name: Yang Dongliang

Organization: Department of infection, Union Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology

Tel：027-85726114

E-mail：dlyang@tjmu.edu.cn

Technical cooperation organization

Organization： National Institutes for Food and Drug Control（NIFDC）

rofessional department：Hepatitis enterovirus vaccine room

Address：No.31 Huatuo Road, Daxing District, Beijing

Name: Hu zhongyu Tel：010-53851772 E-mail：huzy67@163.com
statistic organization
Organization：Beijing Kangterite Statistical Technology Co., Ltd Address: Room 1018w, Sihui building, Huihe South Street, Chaoyang District, Beijing Postcode:100023 Manager:Jiang zhiwei Tel:18618483152 E-mail：zhi.wei.jiang@ktstat.com
Data management organization
Organization：Beijing Kangterite Statistical Technology Co., Ltd Address: Room 1018w, Sihui building, Huihe South Street, Chaoyang District, Beijing Postcode:100023 Manager:Li Guijuan Tel: 13601034495 E-mail：gui.juan.li@ktstat.com
Contract Research Organization(CRO)
Organization name: Chongqing Medleader Bio-Pharm Co., Ltd Project manager and contact information Project manager:Liu Yan Address: 669 Maliu Avenue, Mudong Town, Banan District, Chongqing Postcode:401338

Tel: 023-68179162 E-mail： liuyan@medleader.com.cn
Organization name: Beijing Stamexcel Technology Co., Ltd Address: 15 / F, block A, 21st century building, 40 Liangmaqiao Road, Chaoyang District, Beijing Postcode:100020 Project manager and contact information Project manager:Song Hong Address: 15 / F, block A, 21st century building, 40 Liangmaqiao Road, Chaoyang District, Beijing Postcode:100020 Tel: 13701357899 E-mail：sh@stemexcel.com

Programme summary

Study title	Safety and tolerability of a Recombinant Novel Coronavirus Vaccine (CHO cells): a multi-center, double-blind, randomized, placebo-controlled parallel phase Ⅰ clinical trial in healthy volunteers aged from 18 to 59 years old
Brief title	Recombinant Novel Coronavirus Vaccine (CHO cells) Phase I clinical trial
Products specifications	Each dose contains 25 μ g / 0.5ml / bottle (low dose), 50 μ g / 0.5ml / bottle (high dose)
Indication	Prevent respiratory diseases caused by novel coronavirus infection
Study population	Volunteers aged 18 to 59 years old
Principal investigators	Research center 1(Leading center): Hong Ren, Xian Yu Research center 2: Lihong Liu
Study Purpose	**Main purpose:** To evaluate the safety and tolerability of recombinant novel coronavirus vaccine (CHO cells) in healthy volunteers aged from 18 to 59 years old . **Secondary purpose:** To explore the immunogenicity of recombinant novel coronavirus vaccine (CHO cells) preliminarily.
Study design	**Overall design:** A multicenter, double-blind, randomized, placebo-controlled parallel clinical trial design.
	Study population: 50 healthy volunteers aged 18-59(including 18 and 59 years old), including

	both male and female. **Research Plan and Implementation:** Dose: low-dose group (25 μ g / 0.5ml) and high-dose group (50 μ g / 0.5ml); Immunization program: 0, 1, 2 months; In the first stage, the subjects enrolled were randomly divided into low-dose group (20 cases) and placebo group (5 cases); Seven days after the first dose of vaccination in the first stage, DSMB conducted a safety assessment and agreed to undergo the second stage study. In the second stage subjects in the high-dose group (20 cases) and placebo group (5 cases) were enrolled; After each dose of vaccination, the subjects will be followed up for 30 days. In case of suspension / termination criteria events happen, DSMB will decide whether to inoculate the next dose; In other cases, it is up to the investigator to decide whether to inoculate the next dose; Each dose was observed in the hospital for 1.0 h before leaving the hospital. The safety of each subject was observed until one year after the whole vaccination. **Safety assessment：** **AE&SAE：** All adverse events (AE) 30 minutes after each dose of vaccination, all AEs from 0-7 days, and all AEs from 8-30 days were collected. All AEs from the first dose of vaccination to 30 days after the whole vaccination, and all serious adverse events (SAEs) within 1 year after the first dose of vaccination were collected. Vital signs and physical examination: All subjects were tested for axillary temperature during the screening period, before each dose of vaccination, every day from the first dose of vaccination to the 30th day after the whole vaccination, at 6th month and 12th month after the whole vaccination. Blood pressure and pulse were checked during the screening period, before each dose of vaccination, and on the 4th and 7th days after each dose of inoculation; All the subjects were checked for physical examinations (skin and mucous

	membrane, lymph nodes, head, neck, chest, abdomen, spine / limbs) in screening period, before each dose of vaccination and on the fourth day after each dose of vaccination. **Laboratory indicators:** All the subjects were tested for blood biochemistry (alanine aminotransferase, aspartate aminotransferase, total bilirubin, urea, creatinine, fasting blood glucose), blood test (white blood cell, platelet, hemoglobin, lymphocyte) and urine test (urine protein, urine red blood cell) in the screening period, before and 4 days after each dose of inoculation. The test of blood biochemistry (creatine kinase and lactate dehydrogenase) was increased before and 4 days after the third dose of inoculation. For age of female on fertility blood pregnancy test should be carried out in screening period, and urine pregnancy test should be conducted before each dose of vaccination. SARS-Cov-2 IgM and IgG antibodies were detected in all subjects during the screening period. SARS-Cov-2 was detected by real-time RT-PCR in screening period, before each dose and 1 year after inoculation. If the test items above have abnormal items that are above grade 1 as judged by researchers or clinically significant by clinicians, they should be retested in time and followed up closely. **Immunogenicity test：** For all the subjects, about 30 ml blood samples are collected for cellular immune test before each dose of vaccination, 14 days after the first dose of vaccination, 7 days after the second dose of vaccination, 7 days after the third dose of vaccination, and 1 and 6 months after the whole course of vaccination. For all the subjects, about 8 ml blood samples are collected for humoral immune test before each dose of vaccination, 14 days after the first dose of vaccination, 7 days after the second dose of vaccination, 7 days after the third dose of vaccination, and 1 month and 6 months after the whole vaccination.
	Safety data monitoring:

	DSMB was set up to monitor the safety data during the study. 1) DSMB is composed of 3 experts, including 1 independent clinical medicine, 1 epidemiology and 1 statistics expert. 2) DSMB is responsible for monitoring the safety data during the study. 3) The clinical implementation team is responsible for timely and accurate uploading of data to eCRF. Seven days after the first dose of low-dose group is inoculated, the safety data is submitted to DSMB experts for review. The DSMB experts make decisions and give suggestions for the sponsor to decide whether to continue the next stage (the first dose of high-dose group). 4) All safety data of high dose group were submitted to DSMB experts for review 7 days after the first dose of vaccination. 5) Seven days after the whole vaccination for high-dose group, all safety data are submitted to DSMB experts for review. 6) In case of safety problems during vaccination (e.g. meeting the criteria for suspension / termination of trials specified in the protocol), DSMB will hold an emergency meeting to assess and report to the ethics committee.
Inclusion criteria	1) According to the observation age of this clinical trial: 18-59 years old (including 18 and 59 years old) adults; 2) The subjects voluntarily agreed to participate in the study, signed the informed consent, and provided valid identification; they understood and complied with the requirements of the trial protocol; they were able to take part in an one-year follow-up; 3) The temperature of armpit was less than 37.3 ℃; 4) The body mass index (BMI) ranged from 18-28kg/m²(including 18 and 28 kg/m²); 5) Women of childbearing age and men agreed to take effective contraceptive measures during the study.

Exclusion criteria	1）The vital signs, physical examination and laboratory test indexes specified in the protocol were abnormal, which were judged by clinicians to be of clinical significance; 2）Have a history of severe allergy to any component of the study vaccine, including aluminum preparation, such as anaphylactic shock, anaphylactic laryngeal edema, allergic purpura, thrombocytopenic purpura, local anaphylactic necrosis reaction (Arthus reaction); or have a history of severe adverse reactions to any vaccine or drug, such as allergy, urticaria, skin eczema, dyspnea, angioneuroedema, etc; 3）Patients with a history of SARS and COVID-19, or meet any of the following criteria: ①previous infection or onset history of SARS-CoV and SARS-CoV-2; ②contact history with confirmed patients / suspected patients during the epidemic period of SARS-CoV-2; ③positive results of IgM and / or IgG antibodies to sars-cov-2; ④positive results of real-time RT-PCR nucleic acid detection of SARS-CoV-2； 4）Antipyretic or analgesics were taken within 24 hours before the first dose of vaccination； 5）The subunits vaccine and inactivated vaccine were inoculated 14 days before the first dose of vaccination, and the attenuated live vaccine was inoculated within 30 days； 6）Patients with the following diseases: ①Acute febrile diseases； ②Digestive system diseases (such as diarrhea, abdominal pain, vomiting, etc.) in the past 7 days； ③With congenital malformations or developmental disorders, genetic defects, severe malnutrition, etc.； ④Congenital or acquired immune deficiency or autoimmune disease history or receiving immunomodulator treatment within 6 months, such as hormone; or

	monoclonal antibody; or thymosin; or interferon; but local medication (such as ointment, eye drops, inhalants or nasal spray) is allowed, and local medication should not exceed the dosage recommended in the instruction manual or have any signs of systemic exposure； ⑤Chest CT examination was judged by researchers to be clinically significant or any positive results for HBsAg, HCV antibody, HIV antibody or syphilis specific antibody； ⑥ Neurologic disease or neurodevelopmental dysplasia (such as migraine, epilepsy, stroke, seizures in the last three years, encephalopathy, focal neurological deficit, guillain-barre syndrome, encephalomyelitis or transverse myelitis); history of mental illness or family history； ⑦Functional asplenia and splenectomy due to any reason； ⑧ Patients with severe chronic diseases or disease in progress can not be smoothly controlled, such as diabetes, thyroid disease; ⑨Severe liver and kidney diseases; respiratory diseases that currently require routine drug treatment (e.g., chronic obstructive pulmonary disease [COPD], asthma) or any treatment that has aggravated respiratory diseases (e.g., exacerbation of asthma) in the last five years; history of severe cardiovascular diseases (such as congestive heart failure, cardiomyopathy, ischemic heart disease, arrhythmia, conduction block, myocardial infarction) Pulmonary heart disease) or myocarditis or pericarditis； ⑩Thrombocytopenia, coagulation dysfunction or anticoagulant therapy, etc.； ⑪Cancer patients； ⑫Have received blood or blood related products within 3 months, including immunoglobulin; or have planned to use them during the study period； 7）Lactation women or pregnant women (including positive urine pregnancy test)； 8）Use any research or unregistered products (drug, vaccine, biological

	product or device) other than the research product within 3 months, or plan to use them during the study period； 9）Dizzy with blood or dizzy with needle； 10）The investigators believe that the presence of any disease or condition in the subjects may put them at an unacceptable risk; the subjects cannot meet the requirements of the protocol; and the evaluation of vaccine response is interfered with.
Study endpoint	**Safety endpoint：** 1. The incidence of all AEs from the first dose of vacciantion to one month after the whole vaccination 1)Incidence of total AEs; 2) The incidence of AEs related to the study vaccine; 3) The incidence of level 3 and above AEs; 4) The incidence of level 3 and above AEs related to the study vaccine; 5) The incidence of AEs leading to quit； 6) The incidence of AEs related to the study vaccine leading to quit; 2. The incidence of all serious adverse events (SAEs) and vaccine related SAEs within 1 year after the first dose of vaccination to the whole course of vaccination; 3. The clinical significance changes of laboratory test indexes after each dose of inoculation compared with that before the first dose of inoculation. Laboratory test index: √Blood routine examination: white blood cell, platelet, hemoglobin, lymphocyte; √Blood biochemistry: alanine aminotransferase, aspartate aminotransferase, total bilirubin, urea, creatinine, fasting blood glucose (creatine kinase and lactate dehydrogenase were increased before and 4 days

	after the third dose of inoculation); √Urine routine: urine protein, urine red blood cell. **Immunogenicity endpoint:** Cellular immunity: For all subjects, the levels of IL-2, IL-4, IL-5, IL-6 and IFN - γbefore each dose of vaccination, 14 days after the first dose of vaccination, 7 days after the second dose of vaccination, 7 days after the third dose of vaccination, and 1 month and 6 months after the whole vaccination. Humoral immunity: For all subjects, the positive rates of novel coronavirus (SARS-CoV-2) neutralizing antibody, S protein binding antibody (IgG) and RBD protein binding antibody (IgG) before each dose of vaccination, 14 days after the first dose of vaccination, 7 days after the second dose of vaccination, 7 days after the third dose of vaccination, and 1 months and 6 months after the whole vaccination. For all subjects, the novel coronavirus (SARS-CoV-2) neutralizing antibody and S protein binding antibody (IgG), RBD protein binding antibody (IgG) titer level and the fold increase relative to pre-vaccination before each dose of vaccination, 14 days after the first dose of vaccination, 7 days after the second dose of vaccination, 7 days after the third dose of vaccination, and 1 month and 6 months after the whole vaccination.
Study hypothesis	Not applicable
Sample size calculation	According to the requirements of "*Technical guidelines for clinical trials of vaccines*": phase I clinical trial is a small-scale study (20-30 volunteers). In this trial, high and low doses were used, and placebo parallel control was used to observe the clinical tolerability. The total sample size was 50 cases.
Suspension or termination criteria	This study or any other study has obtained new data on the vaccine for the study, and the administration, the sponsor, the researcher, and / or the IRB propose to suspend / terminate the trial. Suspension criteria:

<table>
<tr><td></td><td>In case of any of the following conditions in any cohort, the trail shall be suspended and immediately reported to the ethics committee, the relevant departments of the provincial and State Drug Administration, and DSMB expert meeting shall be held for safety demonstration analysis to determine whether to continue the trail.

<table><tr><th>Events that cause a pause</th><th>Number of cases /%</th></tr><tr><td>Vaccine related deaths or serious life-threatening adverse events occurred during the study</td><td>≥1 case</td></tr><tr><td>Adverse events ≥ grade 3 and lasting for 48 hours after any dose of vaccination; and / or abnormal laboratory indicators with level ≥ grade 3 after any dose of vaccination</td><td>>15% of vaccinated population</td></tr></table>
Termination criteria:
In case of the following conditions in any cohort, the trial should be terminated and immediately reported to DSMB, ethics committee, provincial and State

<table><tr><th>Events leading to termination</th><th>Number of cases /%</th></tr><tr><td>Adverse events ≥ grade 3 and lasting for 48 hours after any dose of vaccination; and / or abnormal laboratory indicators with level ≥ grade 3 after any dose of vaccination</td><td>>30% of vaccinated population</td></tr></table>Drug Administration.</td></tr>
<tr><td>Related definitions</td><td>1) "Month" in the follow-up is defined as "30 days"; year is defined as 365 days;
2) At the age of female on fertility: refers to the women in the specific period from the maturity of female reproductive organs (menarche) to ovarian failure</td></tr>
</table>

	(menopause); 3) SAE: in the process of clinical trial, events such as hospitalization, prolonged hospital stay, disability, impact on work ability, life-threatening or death, and congenital malformation occurred; 4) 18-59 years old: over 18 years old (i.e. the day of 18 years old), less than 60 years old (that is, the day of 60 years old birthday); 5) Day x: in this protocol, the vaccination day is 0 days. For example, the vaccination day (V2) is 10:00 a.m. on Monday, 10:00 a.m. of this Friday is the starting point of the fourth day (V3, i.e., V2+4), and 10:00 a.m. of next Monday is the starting point of the seventh day (V4, i.e., V2+7).
Study duration	Each subject participates in the study for about 14 months (for 3 doses). The total duration of this study was about 15 months.
Statistical analysis strategy	After all subjects completed the whole course of vaccination for 1 month and 1 year of safety observation, statistical analysis was conducted respectively.

Safety and tolerability of a Recombinant Novel Coronavirus Vaccine (CHO cells): a multi-center, double-blind, randomized, placebo-controlled parallel phase Ⅰ clinical trial in healthy volunteers aged from 18 to 59 years old

1. Brief introduction

The recombinant novel coronavirus vaccine (CHO cell) is a biological product developed by Anhui Zhi Fei Long Ke Ma Biological Pharmaceutical Co., Ltd., which belongs to the 1 category of biological products, and is an unlisted innovative vaccine both at home and abroad. The novel coronavirus vaccine (CHO cell) is being implemented according to the Provisions for drug registration, Good Clinical Practice and the guidance principles for the quality management of clinical trials of vaccines. The test dose of novel coronavirus vaccine (CHO cells) includes two doses of high dose (50 g/ 0.5mL/ bottle) and low dose (25 g/ 0.5mL/ bottle). The subjects were healthy people aged 18-59 years. Clinical research institutions registered in the "drug clinical trial institution record management information platform" were selected for clinical research. The research units were equipped with emergency equipment and drugs on site, the medical staff should have professional training and be able to properly handle various emergencies, adverse events (AE) and serious adverse events (SAE) according to GCP requirements, and clearly identify them in the original cases. The novel coronavirus vaccine (CHO cell) was evaluated in 18-59 healthy people at the age of 18-59, and the immunogenicity was preliminarily evaluated.

2．Study objective

Main purpose:

Evaluation of the safety and tolerance of novel coronavirus vaccine (CHO cell) in 18 to 59 years old healthy population

Secondary purpose:

Preliminary exploration of novel coronavirus vaccine (CHO cell) immunogenicity.

2.1 RELATED DEFINITIONS

1) "Month" is defined as "30 days"; year is defined as 365 days;

2)At the age of female on fertility: refers to the women in the specific period from the maturity of female reproductive organs (menarche) to ovarian failure (menopause);

3) SAE: in the process of clinical trial, events such as hospitalization, prolonged hospital stay, disability, impact on work ability, life-threatening or death, and congenital malformation occurred;

4) 18-59 years old: over 18 years old (i.e. the day of 18 years old), less than 60 years old (that is, the day of 60 years old birthday);

5) Day x: in this protocol, the vaccination day is 0 days. For example, the vaccination day (V2) is 10:00 a.m. on Monday, 10:00 a.m. of this Friday is the starting point of the fourth day (V3, i.e., V2+4), and 10:00 a.m. of next Monday is the starting point of the seventh day (V4, i.e., V2+7).

3. Clinical trial institutions and sites

Responsible unit 1: the Second Affiliated Hospital of Chongqing Medical University, formerly known as Chongqing Kuanren hospital, which was founded in 1892. With the concept of "*care, focus and innovation*", the hospital is a Grade A hospital integrating medical treatment, teaching, scientific research, prevention and health care. The hospital has two hospital districts (Yuzhong hospital district and Jiangnan hospital district), with 2580 beds and more than 3500 open beds. The annual number of outpatients is 1.372 million, and more than 76000 inpatients are admitted.

There is a complete set of departments, including 43 clinical and medical technical departments. Internal medicine (infectious diseases) and neurology are national key disciplines, cardiovascular medicine, respiratory medicine, nephrology departmen, medical imaging department (including radiation department, ultrasound department, nuclear medicine department) and infectious disease department are national key clinical specialties, obstetrics and Gynecology, laboratory, general surgery (including hepatobiliary surgery) Department of geriatrics, Gastroenterology, Gastroenterology and ophthalmology are the key clinical specialties in Chongqing, the rehabilitation department of traditional Chinese medicine is the key specialty of the 12th Five Year Plan of the National Administration of traditional Chinese medicine, and the Department of spleen and stomach disease of traditional Chinese medicine is the key specialty of Chongqing in the 12th Five Year Plan. The first-class discipline of clinical medicine is a provincial key discipline, and its 17 secondary disciplines are all provincial key disciplines; medical imaging, rehabilitation medicine and physiotherapy, internal medicine (infectious diseases) and obstetrics and gynecology are the key medical

disciplines in Chongqing. There are 13 Chongqing clinical diagnosis and treatment research centers and 10 Chongqing medical quality control centers.

The hospital has three provincial and ministerial research platforms, including the Key Laboratory of molecular biology of infectious diseases of the Ministry of education, Chongqing Key Laboratory of ultrasound molecular imaging and Chongqing research center of reproductive development and stem cell therapy engineering technology, and two regional medical clinical research centers of Chongqing infectious diseases and geriatrics. It is a national drug clinical and medical device clinical trial institution with 14 specialties Industry group, is the national stem cell clinical research institute.

It has set up an independent ethics committee, which is in charge of the director of the ethics committee and has an office under it. The ethics committee is composed of the hospital's Medical Pharmacy expert group, external professional lawyers and community representatives, and is responsible for the medical ethics review of the drug clinical trials undertaken by the professional group.

Responsible unit 2: Beijing Chaoyang Hospital , Capital Medical University

Beijing Chaoyang Hospital , Capital Medical University was established in 1958. It is the Third Clinical Medical College of Capital Medical University and the hospital of Beijing respiratory disease research institute. It is a Grade-A hospital in Beijing which integrates medical treatment, teaching, scientific research and prevention. The hospital is now a hospital with three sites, with a total area of 102800 square meters and a construction area of 21000 square meters. There are more than 4300 employees. There are 1900 beds, 59 clinical and medical technical departments, complete disciplines and strong comprehensive treatment ability. The annual number of outpatient and emergency departments is about 3.8 million, and the number of hospitalized patients is more than 89000, and the number of operations is about 34000.

It has 1.5T and 3.0T magnetic resonance imaging system, PET-CT, super high-end dual source CT, all digital linear accelerator, digital subtraction angiography X-ray machine, digital color ultrasonic diagnostic instrument, flat-panel digital gastrointestinal radiography machine, automatic biochemical assembly line and other medical equipment.

The respiratory disease discipline of the hospital is a national key discipline. At the same time, it has eight national key clinical specialties, including intensive care medicine,

anesthesia, cardiovascular medicine, occupational disease, clinical nursing, respiratory medicine, emergency medicine and laboratory. The hospital attaches great importance to discipline construction and research platform construction, and has established a research center with international advanced level, which provides a platform for translational medicine research in the whole hospital. In recent years, our hospital has successively undertaken national key scientific and technological projects, 863 plan, 973 Plan, major special projects of the Ministry of science and technology, key international cooperation projects, National Natural Science Foundation of China, national emergency science and technology action project of SARS prevention and control, Ministry of health and other scientific research projects and subjects at all levels.

The national drug clinical trial institution of Beijing Chaoyang Hospital was established in December 1999. In 2007, 2013 and 2017, the National Medical Products Administration (NMPA) institution qualification certification and review inspection were successfully passed. At present, 28 specialties have been approved for drug clinical trials, and 50 departments have completed the filing of clinical trials of medical devices. The number of clinical trial specialties is large, with a wide range of categories and outstanding comprehensive strength. It can undertake the clinical trials of drugs and medical devices in phases I to IV.

Beijing Chaoyang Hospital has set up an independent ethics committee, which is responsible for medical ethics review of clinical trials undertaken by professional groups and clinical studies initiated by researchers. The ethics committee successfully passed the SIDCER-FERCAP certification on September 12, 2015, and became one of the members of FERCAP.

Site 1: phase I clinical laboratory of the Second Affiliated Hospital of Chongqing Medical University

The Second Affiliated Hospital of Chongqing Medical University was the first batch of clinical pharmacology bases approved by the Ministry of health in 1983. It was renamed as a drug clinical trial institution in 2006. It has been identified or reviewed by the China Food and Drug Administration for six times. The institutions are national drug clinical trial institutions, national medical device clinical trial institutions and national stem cell clinical research institutions. The president of the Institute is the director of the institution. There are full-time

office directors, secretaries (part-time drug administrators), quality controllers and Data Administrators of the agency office. There are 15 professional groups in the institution, and 104 drug clinical trials have been carried out in recent five years. The laboratory area is 600 square meters, equipped with a variety of special testing equipment, which can meet the requirements of auxiliary examination of drug clinical trials. In addition to complete hardware facilities, the center also specified detailed institutional management system and SOP according to GCP principles to standardize and guide clinical trials.

Phase I ward in Yuzhong District is designed to cover 39 beds. It is constructed and managed in strict accordance with the requirements of GCP. It has a good experimental and working environment. Phase I ward of Jiangnan hospital area under construction covers an area of about 1500 square meters, and more than 60 beds are designed and opened, which will be put into use soon.

The 24-hour closed management is implemented in the test area, equipped with security and access control system, HD monitoring system, cold chain management system, etc. The experimental area consists of general observation room, rescue observation room, subject activity room, catering room, informed consent room, drug storage room, sample processing / storage room, archives room, doctor's office, duty room, screening room / inspector's integrated office and other functional areas. The structure of the research team in phase I clinical trial research room is reasonable, including research doctors, research nurses, research pharmacists, laboratory administrators, drug administrators, sample managers, full-time quality controllers. All the researchers have passed GCP and clinical trial related professional skills training and obtained certificates. Ren Hong, the principal researcher, is the president of the Second Affiliated Hospital of Chongqing Medical University, the chief expert of the national major infectious diseases project, and a member of the 13th CPPCC National Committee. He is the leader of internal medicine (infectious diseases) of national key discipline of Chongqing Medical University, director of Viral Hepatitis Research Institute of Chongqing Medical University and director of Key Laboratory of molecular biology of infectious diseases of Ministry of education. He has been engaged in the research on immune pathogenesis and clinical outcome of viral hepatitis for a long time. He has successively presided over and undertaken the national science and technology major projects of infectious diseases in the 11th five year plan, the 12th Five Year Plan and the 13th five year plan, and the Yangtze River scholars and innovation team development plan of the Ministry of

education. In the past 10 years, as a principal investigator, she has undertaken more than 20 clinical trials of drugs, including therapeutic hepatitis B vaccine. Yu Xian, head of phase I clinical laboratory, has educational background and research experience in clinical medicine, pharmacology, immunology and clinical pharmacy. She has participated in the research and development of mumps inactivated vaccine and EV71 inactivated vaccine. She has been committed to the research and development of pathogenic microorganism vaccine, clinical research of comprehensive prevention and treatment of bacterial infection, and drug PK / PD research of special population. More than 20 research papers including vaccine have been published as the first author or corresponding author. As the person in charge, she presided over 8 scientific research projects at all levels including the National Natural Science Foundation of China. As the first inventor, she was granted a national invention patent (DNA vaccine). As a principal investigator, she undertook a number of phase 1 and be clinical trials.

In recent years, the center has carried out 4 therapeutic vaccine trials and 2 interferon projects for immune system. Two therapeutic double plasmid HBV DNA vaccine phase II projects were started in 2016. The vaccines used in the project are the original class 1 therapeutic biological products. The implementation process of the project is complex. More than 20 centers in China have carried out the project, and the center, as the leader unit, coordinates the simultaneous development of various units. During the experiment, all volunteers were tested in strict accordance with the relevant laws and regulations of the scheme. During the follow-up observation, the safety of the subjects was strictly protected, and the emergency measures were complete. During the test, experts invited by the sponsor were also accepted for inspection, and no major problems and findings were found. The overall quality of the project was good.

Site 2: Phase I clinical laboratory of Beijing Chaoyang Hospital

The phase I clinical laboratory of Beijing Chaoyang Hospital passed nmpa qualification certification in June 2006, and passed the follow-up review certification continuously. There is one phase I ward and one analysis laboratory.

The phase I ward is constructed and managed in strict accordance with the requirements of GCP. It has a good experimental environment and working environment. There are 25 special beds in total. It is equipped with observation room, treatment room, reception room, activity room, duty room and other units. The rescue equipment and drugs are complete, which can fully protect the rights and interests of subjects and meet the needs of phase I

clinical trial. The research room has established a phase I ward document system, including 152 items of ward management system (25 items), work responsibilities (13 items), standard operating procedures (79 items), emergency plan (23 items), diagnosis and treatment routine (12 items), etc., strictly following the NMPA guidelines. Phase I laboratory can carry out pharmacokinetic and safety studies of innovative drugs, bioequivalence and generic drug consistency evaluation, medical device verification, vaccine clinical research, etc. it has undertaken more than 70 phase I clinical studies, and all the projects have passed the verification of nmpa clinical trials.

CNAS quality management system has been established and approved by the analysis laboratory. The analytical laboratory has high-quality software and hardware conditions, and the experimental instruments and equipment are as follows: three LC-MS / MS (5500QTRAP，API5500，API3200MD) mass spectrometer, precision electronic analytical balance, low-temperature refrigerator, low-temperature high-speed centrifuge, nitrogen drying concentration instrument, solid-phase extraction instrument, ultra clean working table and micro sampler (complete specifications), with a total value of more than 20 million yuan, which can meet the requirements of phase I Work needs. The main instruments have operation regulations, maintenance system and special person responsible system, and some instruments and equipment are measured and verified regularly according to relevant national regulations.

In the past five years, the phase I clinical laboratory has been approved as one sub project of major new drug development during the 12th Five Year Plan period, 2 sub projects of major new drug creation during the 13th Five Year Plan period, 1 sub project of 863 project, 11 projects of National Natural Science Foundation of China and 9 projects of Beijing Natural Science Foundation. A total of 56 SCI papers have been published and 6 invention patents have been applied.

Liu Lihong, principal investgator, professor and doctoral supervisor, is currently the chief pharmacist, deputy director of clinical trial institution, director of pharmaceutical affairs department and director of phase I Research Office of Beijing Chaoyang Hospital Affiliated to Capital Medical University. Main research directions: hospital pharmacy, molecular pharmacology, pharmacokinetics, pharmacogenomics, etc. She is also the chief pharmacist of Beijing Municipal Hospital Administration; member of the Standing Committee of clinical pharmacy of Chinese Medical Association; chairman of Pharmaceutical Service Committee of

Chinese Pharmaceutical Association; executive director of China Women's Doctor Association and chairman of pharmaceutical professional committee; She is a member of BMJ International Advisory Committee, chairman of applied and translational Pharmacy Committee of Beijing Pharmaceutical Association, vice chairman of Clinical Pharmacy Committee of Beijing Medical Association, executive director of Beijing Pharmaceutical Association, director of Beijing clinical pharmacology Committee, vice president of Beijing Pharmacist Association, and vice president of World Federation of Chinese medicine associations. She has undertaken more than 20 projects of GCP platform of the Ministry of science and technology during the 12th Five Year Plan and the 13th five year plan, major new drug creation, 863 project, NSFC, and more than 70 phase I clinical studies. Over the past five years, more than 120 articles and more than 50 SCI articles have been published in core journals. She has won the "May 1st Medal", "March 8th red flag bearer", "top 10 harmonious contribution women in China", "excellent medical workers in the capital", etc. Since 2004, she has participated in the training courses of national practice for quality management of clinical trials (GCP) and obtained the certificate. Participated in the training of national GCP inspectors and was seconded to nmpa Center for Drug Evaluation,NMPA.

4. Clinical trial management

4.1 Clinical trial parties and responsibilities

4.1.1 SPONSOR

1) Provide the preliminary clinical trial protocol, review and approve the final clinical trial protocol, informed consent form, electronic case report form (eCRF) and data management plan, etc;

2) Provide clinical trial notice and investigator's brochure, including field application documents of chemistry, pharmacy, toxicology, pharmacology and clinical data (including completed and ongoing) of the research vaccine;

3) Provide experimental vaccine for clinical research and issue verification report;

4) Provide placebo for clinical research and issue verification report;

5) Responsible for the safe storage and transportation of the vaccines (including trial vaccines and placebo);

6) Assign full-time personnel to be responsible for the monitoring of clinical trial safety

information and the management of SAE report, master the latest status of safety information of the whole clinical trial, and timely report to all participating researchers and regulatory authorities;

7) Participate in the investigation and treatment of adverse reactions and AE, and provide medical treatment or related compensation for adverse reaction cases and AE cases with clinical proof related to vaccine according to relevant regulations;

8) Be responsible for sending qualified inspectors or entrusting contract research organizations to evaluate and select clinical trial sites, perform supervision duties according to GCP requirements during the trial, and verify research data;

9) Organize the audit of clinical trials to ensure the quality of clinical trials, ensure that the clinical trials are conducted in accordance with GCP and protocol requirements, and be ultimately responsible for the quality of clinical trials;

10) Establish an independent Data and Safety Monitoring Board(DSMB);

11) Provide funding for clinical research.

4.1.2RESPONSIBLE ORGANIZATIONS

1) Participate in the formulation of clinical trial protocol and organize the implementation of clinical trial procotol;

2) Assist in the preparation and review of clinical trial related documents such as informed consent form, original medical records, eCRF and contact card;

3) Submit ethical review materials to ethics committee and obtain approval certificate;

4) Establish the organization management system and quality management system of vaccine clinical trial, write SOP and conduct training;

5) The institution is put on record in the "drug clinical trial institution record management information platform";

6) Has management mechanism and measures to prevent and deal with emergencies in vaccine clinical trials, and has SAE emergency response expert team and technical ability to deal with SAE;

7) Responsible for epidemic safety training of clinical trial personnel;

8) Ensure the safe storage and use of research vaccines and manage biological samples;

9) Organize on-site recruitment and enrollment of subjects, organize on-site vaccination, and supervise the implementation of on-site work;

10) To organize the follow-up of subjects and AE collection, and to organize the report, investigation and treatment of AE;

11) Organize to complete all forms and ECRF in the test site;

12) According to the requirements of GCP, manage and save the clinical trial related data, and confirm the filing of the trial data;

13) Accept the inspection, inspection and on-site verification of the third party (the sponsor, the National Bureau, and the Provincial Bureau);

14) Issue clinical trial summary report.

4.1.3 SITES

1) Set up qualified field researchers team, establish environmental facilities to meet the requirements of clinical trials, and assist the responsible institutions in filing;

2)Recruite and enroll the subjects who met the requirements of clinical trial protocol;

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

4) Deal with AE, deviation and violation of protocol during the study, and report SAE, deviation and violation of protocol as required;

5)Collect the original data of clinical trials and input into eCRF;

6) Manage research vaccines and biological samples according to GCP requirements;

7) Accept the audit, inspection and on-site verification of the third party (the sponsor, the National Bureau, and the Provincial Bureau);

8) Manage and keep the test related data according to GCP requirements until 5 years after the completion of the clinical trial.

4.1.4 DSMB

Data And Safety Monitoring Board (DSMB)

Responsibilities of DSMB:

DSMB was set up to monitor the safety data during the study.

1) DSMB is composed of 3 experts, including 1 independent clinical medicine, 1 epidemiology and 1 statistics expert.

2) DSMB is responsible for monitoring the safety data during the study.

3) The clinical implementation team is responsible for timely and accurate uploading of data to ECRF. Seven days after the first dose of low-dose group is inoculated, the safety data is submitted to DSMB experts for review. The DSMB experts make decisions and give suggestions for the sponsor to decide whether to continue the next stage (the first dose of high-dose group).

4) All safety data of high dose group were submitted to DSMB experts for review 7 days after the first dose of vaccination.

5) Seven days after the whole vaccination for high-dose group, all safety data are submitted to DSMB experts for review.

In case of safety problems during vaccination (e.g. meeting the criteria for suspension / termination of trials specified in the protocol), DSMB will hold an emergency meeting to assess and report to the ethics committee.

4.1.5 TECHNICAL COOPERATION UNITS

1) Complete the detection of clinical trial samples and issue the test report;

2) Pharmacopoeia method or test kit approved by the State shall be used for the test method, and the reference value of result judgment, test standard and test standard shall be provided, and the method verification certificate shall be provided if necessary; if the test method provided by the vaccine manufacturer is used, the method provider shall be responsible for the verification of the method and provide relevant data for the method validation of the pharmacodynamics testing unit.

3) Provide certification and quality control laboratory related qualification certificates.

4.1.6 STATISTICAL UNIT

1) Responsible for the randomization and statistical analysis of clinical trial protocol;

2) Be responsible for clinical trial randomization;

3) According to the clinical trial plan, write statistical analysis plan;

4) Carry out statistical analysis according to the proposed statistical analysis plan and write statistical analysis report.

4.1.7 DATA MANAGEMENT UNIT

1) According to the requirements of the protocol, the eCRF draft and the revised version are completed, which are jointly reviewed and finalized by the researcher, the sponsor, the statistician and the project manager;

2) According to the protocol and eCRF, make data management plan and data verification plan;

3) Establish, test and revise the database according to SOP, backup the database, upgrade and transfer the version;

4) Perform data cleaning according to the data verification plan, mainly includes: checking missing data, violating scheme verification, time window checking, logic checking, scope checking, SAE consistency checking, etc.; sending online query and clarifying by researchers;

5) Carry out data quality control inspection;

6) Responsible for the medical code of this study, including non solicitation AE (including SAE);

7) To be responsible for the classification of drug combinations in this study;

8) After the data query clarification, complete the data review report; sort out the statistical population division resolution according to the opinions of researchers, sponsors and statisticians; lock the database and deliver the data to the statistician for statistical analysis;

9) Complete data management report;

10) After the completion of the project, SAS database and data management related documents will be engraved on CD and submitted to the sponsor.

4.1.8 CONTRACT RESEARCH ORGANIZATION (CRO)

Clinical trial monitoring was carried out according to GCP, clinical trial protocol and SOP

1) Assist the sponsor to confirm that the clinical trial institution undertaking the trial has the appropriate conditions to complete the trial, including staffing and training, functional zoning such as emergency room, and all laboratories are fully equipped and running well, and have various conditions related to the test;

2) Verify the delivery, storage, distribution, use, return and disposal of the research vaccine in accordance with the requirements of the protocol, control and record, and check the dose change and drug combination of each subject;

3) Confirm that all the subjects signed the written informed consent before the trial, and the selected subjects were qualified;

4) Confirm that the investigators have received the latest version of the investigator's brochure, protocol, all clinical trial related documents and test supplies, and implement them normally according to the requirements of regulations;

5) Verify that the investigators are trained and authorized in writing before participating in the study;

6) Confirm that all data records and reports are correct and complete, all eCRF entries are correct and consistent with the original data, verify that all medical reports, records and documents provided by the researcher are accurate, complete, timely, legible, date and study number, and verify that the correction, addition or deletion of the data is correct, dated and signed by the researcher;

7) Confirm that all AE are recorded and SAE is reported and recorded within the specified time;

8) Confirm that the researcher kept the necessary documents according to GCP requirements, and the test records and documents were updated and kept in good condition in real time;

9) Determine the deviation from the test protocol, SOP, GCP and relevant regulatory requirements in clinical trials, communicate with researchers in a timely manner, and take appropriate measures to prevent the recurrence of deviation;

10) Verify that the withdrawal and loss of follow-up of the selected subjects have been explained in eCRF;

11) The monitor shall make a written report to the sponsor after each visit, and explain the corrective measures taken or to be adopted for the problems found in the audit; truthfully record the follow-up, test and inspection not conducted by the researcher, as well as whether the errors and omissions have been corrected;

12) After the completion of the clinical trial, assist the research institution and sponsor to sort out the documents and submit them to the National Medical Product Administration (NMPA), and assist in the preparation of on-site registration verification.

Please refer to "research team" for the list of participating units of clinical trial.

4.2 Site management

This is a multicenter clinical trial. The Second Affiliated Hospital of Chongqing Medical University was the group leader and Beijing Chaoyang Hospital of Capital Medical University was the participant.

Both of the two research centers have professional researchers and departments, which are qualified for clinical drug trials.The principal investigator is responsible for clinical trial management. Clinical trial researchers, research nurses, quality control personnel, AE investigators and clinical trial related personnel are set up in the research team. The principal investigator is responsible for interpreting clinical trial protocol, organizing GCP and site operation standard procedure training, guiding researchers to carry out vaccine injection according to requirements, observing and following up, reporting and handling AE and other emergency safety incidents, and managing and quality control the whole process of clinical trial.

5. Background and theory

5.1 Background of the disease

Since December 2019, novel coronavirus infected patients with pneumonia have been successively developed in Wuhan, Hubei. With the spread of the disease, other diseases in other regions and overseas have also occurred. By May 23, 2020, the total number of confirmed cases in China has reached 84522, the total number of deaths has reached 4645, and the total number of confirmed cases abroad has reached 5237963. There were 335815

deaths worldwide. The novel coronavirus outbreak was declared a public health emergency in January 31, 2020 by the WHO. In February 11, 2020, novel coronavirus pneumonia was declared by WHO as "COVID-19".

The novel coronavirus infection source is mainly new coronavirus infected patients, and asymptomatic infected persons may also become the source of infection. The main routes of transmission are droplet transmission, contact transmission, fecal oral transmission and respiratory aerosol transmission of different sizes. Based on the current epidemiological survey, the incubation period of the disease is 1-14 days, mostly 3-7 days. The main manifestations of the patients were fever, fatigue and dry cough. A small number of patients with nasal congestion, runny nose, sore throat and diarrhea and other symptoms. Severe patients usually developed dyspnea and / or hypoxemia one week after the onset of the disease. Severe patients developed rapidly into acute respiratory distress syndrome, septic shock, refractory metabolic acidosis and coagulation dysfunction. Judging from the current cases, most patients have a good prognosis and a few patients are critically ill. The prognosis of the elderly and those with chronic underlying diseases is poor. In children, the symptoms were relatively mild.

5.2 Background of pathogens

The novel coronavirus belongs to the coronavirus of beta genus, with capsule and round or oval shape. The diameter is 60-140NM. The genetic characteristics of novel coronavirus are significantly different from those of SARS-CoV (severe acute respiratory syndrome coronavirus) and MERSR- CoV (middle respiratory syndrome coronavirus). The present study shows novel coronavirus and bat SARS like coronavirus (BAT-SL-COVZC45) have a homology of over 85%. The novel coronavirus (SARS-CoV-2) can be found in human respiratory epithelial cells in about 96 hours in vitro, while it takes about 6 days to isolate and culture VERO E6 (HUH-7 and HUH-7 cells). The understanding of the physical and chemical characteristics of coronavirus mostly comes from the study of SARS-CoV and MERSR- CoV. The virus was sensitive to ultraviolet and heat. The virus could be effectively inactivated by 56 ℃ for 30 minutes, ether, 75% ethanol, chlorine containing disinfectant, peracetic acid and chloroform, but chlorhexidine could not.

5.3 Background of research vaccines

Novel coronavirus pneumonia was officially named " COVID-19" by WHO in February 11, 2020. On the same day, the International Committee on virus classification also announced that the new coronavirus causing the disease was officially named "SARS-CoV-2". In the face of the surging epidemic situation, researchers at home and abroad are competing to invest in the research and development of vaccines and start a race against the virus. From January 26 to 27, 2020, the Chinese Center for Disease Control and prevention and the Zhejiang Provincial Center for Disease Control and prevention successively isolated the virus strains of sars-cov-2, which laid the foundation for vaccine research. Like SARS-CoV and MERS-CoV, S protein and RBD contained in SARS-CoV-2 are still the main targets for vaccine development. Worldwide, under the advocacy and funding of the Innovation Alliance for epidemic prevention, vaccine development projects for SARS-CoV-2 have also been announced.

In terms of vaccine types, the school of medicine of Hong Kong University of China and Pasteur Institute of France will respectively improve the existing influenza vaccine and measles vaccine by adding the expression sequence of SAR-SCoV-2S protein to shorten the research and development cycle; The cooperative teams of Johnso SAR-SCoV-2n & Johnson pharmaceutical and Wuhan Bovo biology and GeoVax labs will respectively use their adenovirus and improved poxvirus platforms to develop the virus vector vaccine of SAR-SCoV-2; the University of Queensland in Australia will use its unique "molecular tweezers" vaccine platform to synthesize SAR-SCoV-2 in vitro S protein "clamp" into a natural polymerization state, which is easy to induce neutralizing antibodies in vivo. This research is assisted by GlaxoSmithKline's powerful "adjuvant platform technology" to further enhance the immune response of this subunit vaccine in vivo. Beijing aidivin biological will cooperate with inovio company of the United States to develop and develop SAR-SCoV-2S protein sequence of DNA vaccine "INO-4800". At the same time, more enterprises and scientific research institutions are focusing on the research and development of new mRNA vaccine. This latest technology uses the mRNA encoding virus antigen as the vaccine, which is translated into viral protein after being directly injected or delivered into the body to induce cell and body fluid immune response. It has the characteristics of low immunogenicity, short production process and fast development speed. With the rapid decoding and publication of SAR-SCoV-2 sequence, researchers can quickly screen antigens and design and synthesize a

variety of potential mRNA vaccines of SAR-SCoV-2. At present, the mRNA vaccine research and development companies such as Moderna in the United States and curevac in Germany, as well as Shanghai Siwei Technology Co., Ltd. and Guangzhou Guanhao Biotechnology Co., Ltd., have announced that they will cooperate with scientific research institutes or independently develop the mRNA vaccine of SAR-SCoV-2.

5.4 THEORY OF TRIAL DESIGN

5.4.1 Theory of trial design and control selection

The new recombinant novel coronavirus vaccine (CHO cell) developed by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. is the 1 category of biological products for prevention, and it is an unlisted innovative vaccine both at home and abroad. In the preclinical study, ani mal immunogenicity test was carried out according to the dosage to be used clinically. The im mune effect was good and the safety was good. Therefore, the clinical trial scheme was design ed according to the requirements of clinical trial procotol.

Covid-19 infection is a susceptible disease of the whole age population. Based on the current epidemiological survey, the incubation period is 1-14 days. According to the current cases, the prognosis of the elderly and those with chronic underlying diseases is poor, and the symptoms of children cases are relatively mild. According to the data from the Chinese center for Disease Control and prevention , most cases (77.8%) are between 30 and 69 years old, and the incidence rate of people aged 18 and below is relatively low (2.4% of all reported cases). At the same time, combined with the age classification standard of the elderly in China, the elderly are over 60 years old, and their immunity is weaker than that of healthy adults. In this clinical trial, the age of the target population was set at 18-59 years old, and the safety from the first dose of vaccination to 1 year after the whole vaccination will be observed.

A multicenter, double-blinded, randomized, placebo-controlled design was used.

For specific scheme design and sample size consideration, please refer to "8. Research design" and "13.3 sample size consideration".

5.4.2 DOSE SELECTION BASIS

According to the dose effect relationship analysis of preclinical antigen dose, combined with the research and development experience of researchers and the characteristics of

recombinant subunit vaccine, two doses of the experimental vaccine were determined. Each dose contained 25 μ g / 0.5 ml / bottle (low dose) and 50 μ g / 0.5 ml / bottle (high dose).

5.5 SUBJECT BENEFIT / POTENTIAL RISK

5.5.1 KNOWN POTENTIAL RISKS

The novel coronavirus infection novel coronavirus novel coronavirus infection (COVID-19) may be prevented from participating in this study, and its preventive effect is less than 100%, like any other vaccine. Meanwhile, some people are inoculated with a placebo, which will not produce protection against new coronavirus infection. Therefore, the new type coronavirus will naturally infect during the observation period.

At the same time, in some cases, antibodies play a role in enhancing virus infection in the process of virus infection. They help the virus enter the target cells and improve the infection rate. This phenomenon is called antibody dependence enhancement (ADE). This phenomenon (non novel coronavirus vaccine) was observed in rhesus monkey. Therefore, it is necessary to pay close attention to the suspected COVID-19 cases or confirmed cases in this clinical trial. If the subjects report suspected or confirmed cases during the trial, they should go to a high-level designated hospital for hospitalization.

At the same time, vaccination may have a certain impact on the nervous system, immune system, so in this clinical trial, we need to pay close attention to the related system symptoms.

The potential risk of vaccinating the study vaccine is limited to the common adverse reactions of any vaccine, such as mild pain at the injection site and occasional mild to moderate redness, swelling and induration. Fever, irritation or inhibition, and anorexia may also occur, but are expected to be mild. In general, the remission will disappear without treatment; individual subjects may have strong reactions (such as high fever, allergic reactions, etc.), and the investigators will closely observe and deal with the symptoms.

Blood collection is required during the study, and pain or ecchymosis may occur after blood collection.

5.5.2 KNOWN POTENTIAL BENEFITS

The potential benefit of novel coronavirus infection in COVID-19 may be obtained by vaccination， that is, to prevent the respiratory disease COVID-19 caused by the novel

coronavirus infection. By participating in this registration trial, the subjects will contribute to the launch of the domestics novel coronavirus vaccine and benefit a wider population.

5.5.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

No novel coronavirus novel coronavirus vaccine is available at home and abroad. The potential benefit of the vaccine may be obtained by vaccination, which is to prevent respiratory diseases caused by new coronavirus infection.

The potential risks of the study vaccine are limited to any common adverse reactions of the vaccine, such as pain, redness, swelling and induration at the injection site, as well as fever, irritation or inhibition, and loss of appetite, but are expected to be mild. During the study, investigators will closely monitor the side effects of the vaccine. In case of any side effects or discomfort, the researcher should be informed in time. If the investigators believe that the subjects are unable to tolerate these side effects, the follow-up study vaccination may be stopped or the trial may be terminated, and the safety will be closely followed up.

6. Product characteristics and preclinical studies / laboratory evaluation

6.1 PRODUCT CHARACTERISTICS

The novel coronavirus spike glycoprotein expressed in recombinant CHO cells was purified by adding aluminum hydroxide adjuvant to the receptor binding domain (NCP-RBD). This novel coronavirus vaccine is used to prevent respiratory diseases caused by new coronavirus infection. It is milky white suspension, which can be stratified by precipitation and easy to disperse.

6.2 PRECLINICAL STUDIES / LABORATORY EVALUATION

The recombinant novel coronavirus vaccine (CHO cell) is a new class of 1 new drugs for the prevention of biological products. There is no reference substance and reference material for the quality research of this project. According to the characteristics of the structure and process of the project, the reference materials for the quality research of the stock solution were prepared according to the ICH guiding principles and the requirements of the drug registration and management measures of the State Drug Administration for the prevention of biological products.

The product was prepared by pilot production process according to GMP requirements and corresponding conditions. The repeatability between batches is good and the quality is

stable. The product quality verification is carried out in accordance with *China Biological Products Regulations*, and the quality standards are reviewed and qualified by the Central Inspection Institute. The purity of the monovalent solution and the finished product reached the established quality standard. The purity of protein was higher than 95.0%, and the bacterial endotoxin was less than 10EU / dose. The vaccine titer met the batch standard. There is no potential toxic substance to human body.

6.2.1 STABILITY TEST

The novel coronavirus vaccine (CHO cell) was studied in 6 consecutive batches according to the guideline of drug stability study, including 3 batches of 25 g/0.5mL/ bottles and 3 batches of 50 g/0.5mL/ bottles. The vaccine was unpacked and placed in a suitable open container for forced stability test (shock test, light test and high temperature test), accelerated stability test and long-term stability test.

Among these tests, the shock test is to put the qualified vaccine in the shaking table at 2 ~ 8 ℃ for 28 days, the results meet the requirements. The light test is to put the qualified vaccine under the conditions of 2 ~ 8 ℃ and illumination of 4500lx ± 500lx for 28 days, the results meet the requirements. High temperature test is to put the qualified vaccine under 37 ± 2 ℃ constant temperature for 28 days. The forced stability test is used to judge the stability of the vaccine deviated from the normal storage condition. The results meet the requirements, but it is not recommended to expose to high temperature environment. In addition, the qualified vaccine was tested by accelerated stability test at 25 ± 2 ℃ for 6 consecutive months and long-term stability test at 2 ~ 8 ℃ for 2.5 years. At present, the accelerated test has been completed for 2 month, which is in line with the requirements.

6.2.2 IMMUNOGENICITY

At present, through the research of Balb / c mice, SD rats and cynomolgus monkeys, the levels of IgG antibody, pseudovirus neutralization antibody and true virus neutralization antibody were detected respectively in different immune stages, and the spleen was taken for ELISpot detection. The results showed that after two injections of this vaccine, the antibody titer was very high, and it had a certain degree against pseudovirus and true virus The results showed that the vaccine had good immunogenicity.

6.2.3 SAFETY EVALUATION

The safety evaluation of the vaccine includes: single dose toxicity test in rats, intramuscular injection stimulation test in rabbits, systemic active allergy test in guinea pigs, 4-week repeated dose toxicity dose exploration test in monkeys (including immune and safety pharmacological indexes), 4-week repeated dose toxicity test in rats and 8-week repeated dose toxicity test in monkeys (with immunogenicity, immunotoxicity and safety pharmacology tests) The novel coronavirus vaccine (CHO) has been completed in the single dose toxicity test, rabbit intramuscular injection stimulation test, guinea pig systemic active allergy test and repeated dose toxicity test (including immune and safe pharmacological indexes) in 4 weeks. The 4 week repeated toxicity test has been completed in the interim report. Cell) is safe and reliable, with good immunogenicity, and can support the development of clinical trials. Other experimental data will be submitted in the later stage to comprehensively evaluate the safety of the vaccine.

7. End points of study

7.1 SAFETY END POINTS

1. The incidence of all AE from the first dose to one month after the whole vaccination,including:

1) Total incidence of AE;

2) The incidence of AE related to the study vaccine;

3) The incidence of AE of grade 3 and above;

4) The incidence of AE of grade 3 and above related to the study vaccine;

5) The incidence of AE leading to quit；

6) The incidence of AE related to the study vaccine leading to quit;

2. The incidence of all serious adverse events (SAE) and vaccine related SAE within 1 year after the first dose of vaccine to the whole course of vaccination;

3. The clinical significance changes of laboratory test indexes after each dose of inoculation compared with that before the first dose of inoculation.

Laboratory test index:

√Blood routine examination: white blood cell, platelet, hemoglobin, lymphocyte;

√Blood biochemistry: alanine aminotransferase, aspartate aminotransferase, total bilirubin, urea, creatinine, fasting blood glucose (creatine kinase and lactate dehydrogenase were increased before and 4 days after the third dose of inoculation）;

√Urine routine: urine protein, urine red blood cell.

7.2 IMMUNOGENICITY END POINTS

Cellular immunity: For all subjects, the levels of IL-2, IL-4, IL-5, IL-6 and IFN - γbefore each dose, 14 days after the first dose, 7 days after the second dose, 7 days after the third dose, and 1 month and 6 months after the whole vaccination.

Humoral immunity: For all subjects, the positive rate of novel coronavirus (SARS-CoV-2) neutralizing antibody, S protein binding antibody (IgG) and RBD protein binding antibody (IgG) before each dose, 14 days after the first dose, 7 days after the second dose, 7 days after the third dose, and 1 months and 6 months after the whole vaccination.

For all subjects, the novel coronavirus (SARS-CoV-2) neutralizing antibody and S protein binding antibody (IgG), RBD protein binding antibody (IgG) titer and fold increase relative to pre-vaccination before each dose, 14 days after the first dose, 7 days after the second dose, 7 days after the third dose, and 1 months and 6 months after the whole vaccination.

8.study design

8.1 OVERALL DESIGN:

A multicenter, double-blinded, randomized, placebo-controlled design was used. (Figure 1: N in the figure indicates the number of subjects, the blue arrow indicates the vaccination time point of the low-dose group, the yellow arrow indicates the vaccination time point of the high-dose group, the purple arrow indicates the collection time point of the humoral immune blood sample, and the red arrow indicates the collection time point of the cellular immune blood sample). See the visit follow-up visit for specific time points.

Figure 1 overview of experimental design

8.1.1 SAMPLE SIZE AND GROUPING

Research population:

50 healthy people aged 18-59(including 18 and 59 years old),both male and female.

Grouping

There were 20 patients in the low-dose vaccine group, 20 in the high-dose vaccine group and 10 in the placebo group.

Study cohort and order of enrollment:

In the first stage, the subjects were randomly divided into low-dose group (20 cases) and placebo group (5 cases); Seven days after the first dose of vaccination, DSMB conducted a safety assessment and agreed to the second stage study. Subjects in the high-dose group (20 cases) and placebo group (5 cases) were enrolled; The subjects will be followed up for 30 days. In case of suspension / termination criteria, DSMB will decide whether to inoculate the second dose; In other cases, it is up to the investigator to decide whether to inoculate the second dose; the subjects will receive the third dose of vaccine 30 days after the second dose (In case of suspension / termination criteria events happen prior to the third dose, DSMB will decide whether to inoculate the third dose; In other cases, it is up to the investigator to decide whether to inoculate the third dose). Each dose will be observed in the hospital for 1.0 h

before leaving the hospital. The safety of each subject will be observed until one year after the whole vaccination.

8.1.2 VACCINATION AND FOLLOW-UP

Please refer to the " follow-up visitfollow-up visit" for the specific visit time and content.

8.1.3 SAFETY OBSERVATION

AE and SAE: collect all adverse events (AE) at 30 minutes after each dose, all AE from 0-7 days, and all AES from 8-30 days after each dose. For the specific visit time and content from the first dose of vaccination to 30 days after the whole vaccination, and from the first dose to 1 year after the whole vaccination, please refer to the " follow-up visitfollow-up visit".

- Serious adverse events (SAE)
- Vital signs and physical examination: All subjects are tested for axillary temperature during the screening period, before each dose of vaccination, after the first dose of vaccination to the 30th day after the whole vaccination, and 6 and 12 months after the whole vaccination; and blood pressure and pulse are checked during the screening period, before each dose of vaccination, on the 4th and 7th days after each dose of inoculation; Physical examinations (skin and mucous membrane, lymph nodes, head, neck, chest, abdomen, spine / limbs) were performed in screening period, before each dose of vaccination and on the fourth day after each dose.
- **Laboratory indicators:** Blood biochemistry (alanine aminotransferase, aspartate aminotransferase, total bilirubin, urea, creatinine, fasting blood glucose)(creatine kinase and lactate dehydrogenase were increased before and 4 days after the third dose of inoculation), blood routine (white blood cell, platelet, hemoglobin, lymphocyte) and urine routine (urine protein, urine red blood cell) were detected in the screening period, before and 4 days after each dose of inoculation. For women of childbearing age, blood pregnancy test should be carried out during screening period, and urine pregnancy test should be conducted before each dose of vaccination.
- Sars-Cov-2 IgM and IgG antibodies were detected in all subjects during the screening period. Sars-Cov-2 was detected by real-time RT-PCR in screening period, before

each dose and 1 year after inoculation.

If the above test items have abnormal items that are above grade 1 as judged by investigators or clinically significant judged by clinicians, they should be retested in time and followed up closely.

8.1.4 SAFETY DATA MONITORING:

DSMB was set up to monitor the safety data during the study.

8.1.5 IMMUNOGENICITY OBSERVATION

About 30 ml blood samples are collected from all subjects before each dose of vaccination, 14 days after the first dose of vaccination, 7 days after the second dose of vaccination, 7 days after the third dose of vaccination, and 1 month and 6 months after the whole course of vaccination. The cytokines IL-2, IL-4, IL-5, IL-6 and IFN - γ are detected.

About 8 ml blood samples are collected from all subjects before each dose, 14 days after the first dose, 7 days after the second dose, 7 days after the third dose, and 1 month and 6 months after the whole vaccination for humoral immune test. Analize the novel coronavirus novel coronavirus (SARS-CoV-2) neutralizing antibody, S protein binding antibody (IgG), RBD protein binding antibody (IgG) positive rate, and the new type of coronavirus (SARS-CoV-2) neutralizing antibody and S protein binding antibody (IgG), RBD protein binding antibody (IgG) titer level and their multiple increase compared with pre-immunization..

8.2 RANDOMIZATION AND BLINDING

8.2.1RANDOMIZATION

In this study, the subjects were randomly assigned to the experimental vaccine group or the placebo group according to the 4:1 block randomization method. The randomized statisticians used SAS software to generate the random table of subjects in each dose group. After the successful screening of the subjects, the field researchers who participated in the trial assigned the random number of the subjects (i.e. the study number and also the vaccine number) according to the order of the subjects.

A total of 50 bottles of spare vaccine should be prepared. In each stage, the number of spare vaccine is randomly assigned to the experimental vaccine group or placebo group according to the ratio of 4:1. The randomized statistician use SAS statistical software to

generate the spare vaccine random allocation table. When the research needs to use the reserve vaccine, the researcher logs in the reserve vaccine acquisition system to obtain the corresponding number of the reserve vaccine (the investigator only sees the research number, the real group of the experimental vaccine and the reserve vaccine is introduced into the system in advance, which is always in a blind state throughout the test; when the number of the reserve vaccine is insufficient, it can be added at any time). The study numbers and spare vaccine numbers of subjects in each center are shown in Table 3.

Table 3 study number and reserve vaccine number of each center

stage	study number	reserve vaccine number
Stage1 (including low-dose group and placebo group)	Center1：01-15 Center2: 16-25	Center1：A01-A15 Center2: A16-A25
Stage2 (including low-dose group and placebo group)	Center1：26-40 Center2: 41-50	Center1：A26-A40 Center2: A41-A50

8.2.2 BLINDING

In this study, a double-blind design was used. The randomized statisticians and other blinders were used to blinded the vaccine. The printed vaccine label was pasted on the designated position of each vaccine according to the blind code. Randomized statisticians supervise vaccine blinding and guide blinding operators to label according to the blind code. After the completion of blinding, the blind code should be sealed by the randomized statistician. The whole process of blinding must be recorded in writing. Blinders shall not participate in other related work of this clinical trial, and shall not disclose the blind code to any person participating in the clinical trial.

8.2.3 EMERGENCY UNBLINDING

At the same time, the randomized statisticians prepare emergency letters. Each letter contains a random code for unblinding. Each random code can correspond to any vaccine number. The real group of the vaccine number can be fed back through the online unblinding

system. Each random code represents a chance of unblinding. Only one vaccine number can be unblinded urgently, and then it will be invalid. Emergency letters shall be provided at the site and sent to the site with the blinded vaccine and kept by the person in charge of the site. All emergency envelopes shall be recovered after the completion of the test, and the opening status of the emergency envelopes shall be checked at the blind review meeting.

If serious adverse events occur during the study period, the subject needs emergency rescue or treatment, and it is necessary to know what vaccine the subject is received.After obtaining the oral or written authorization from the principal investigator, the responsible researcher on the test site can decide whether to carry out individual emergency unblinding, and open and read the emergency letters according to the procedure to carry out emergency unblinding(Log in to the online emergency unblinding system with the uncovering random password in the letter, and perform the emergency unblinding according to the prompt operation), and make records; It should then be reported in time (within 24 hours) to the principal investigator, sponsor, inspector, ethics review committee and drug regulatory agency. The subjects with the study number will terminate the trial for shedding treatment, and the researchers will record the reasons for termination in the case report form. The opened emergency letters should be kept properly and returned to the sponsor after the trial.

8.2.4 UNBLINDING

In this experiment, one-time unblinding method was used. After the subjects completed the safety and immunogenicity observation one month after the whole course of vaccination, the database will be locked after the data file is verified to be reliable and correct, and the database will be opened after the database is locked. The treatment group to which each subject belongs will be listed. The results of unblinding were analyzed by statisticians. Unblinding was performed by the principal investigator who kept the blind code, and the records of unblinding were kept.

8.3 REVISION OF TRIAL PROTOCOL

Any modification to this study protocol should be negotiated with and approved by the sponsor. If an agreement is reached on the necessity of the modification, it will be recorded in writing by the sponsor, and the revised version of the study protocol will replace the earlier version. All protocol revisions need to be submitted to the Ethics Committee (EC)/ (IRB), and important revisions (e.g., those affecting the implementation of the study and the safety of the

subjects) need to be approved by the IRB. Management changes that do not affect the design, purpose or safety of the subjects shall be submitted to IRB for filing or rapid review (as required by IRB).

It is the responsibility of the investigator to ensure that any changes to the protocol during the study period are not implemented until the IRB has reviewed and approved the changes, unless it is to eliminate obvious immediate risks to the subjects.

8.4 STUDY PERIOD

Each subject participates in the study for about 14 months (for 3 doses). The total duration of this study was about 15 months.

9. Target population

50 healthy subjects aged 18-59.

20 patients in the low-dose vaccine group, 20 in the high-dose vaccine group and 10 in the placebo group.

In the first and second stages of center 1, 15 people were assigned respectively, including 12 patients in the low-dose vaccine group and 3 in the placebo group; in the second stage, there were 12 patients in the high-dose vaccine group and 3 in the placebo group.

In the first and second stages of center 2, 10 people were assigned to each stage, including 8 in the low-dose vaccine group and 2 in the placebo group; in the second stage, there were 8 patients in the high-dose vaccine group and 2 in the placebo group.

9.1 INCLUSION CRITERIA

1) According to the observation age of this clinical trial: 18-59 years old (including) adults;

2) The subjects voluntarily agreed to participate in the study, signed the informed consent form, and provided valid identification; they understood and complied with the requirements of the trial protocol;they were able to take part in a one-year follow-up;

3)The axillary temperature was less than 37.3 ℃;

4)The body mass index (BMI) ranged from 18-28kg/m²(inclusive);

5)Women of childbearing age and men agreed to take effective contraceptive measures

during the study.

9.2 EXCLUSION CRITERIA

1) The vital signs, physical examination and laboratory test indexes specified in the protocol were abnormal, which were judged by clinicians to be of clinical significance;

2) Have a history of severe allergy to any component of the study vaccine, including aluminum preparation, such as anaphylactic shock, anaphylactic laryngeal edema, allergic purpura, thrombocytopenic purpura, local anaphylactic necrosis reaction (Arthus reaction); or have a history of severe adverse reactions to any vaccine or drug, such as allergy, urticaria, skin eczema, dyspnea, angioneuroedema, etc;

3) Patients with a history of SARS and COVID-19 ,or meet any of the following criteria: ① previous infection or onset history of SARS-CoV and SARS-CoV-2; ② contact history with confirmed patients / suspected patients during the epidemic period of SARS-CoV-2; ③ positive results of IgM and / or IgG antibodies to sars-cov-2; ④ positive results of real-time RT-PCR nucleic acid detection of SARS-CoV-2；

4) Antipyretic or analgesics were taken within 24 hours before the first dose of vaccination；

5) The subunits vaccine and inactivated vaccine were inoculated 14 days before the first dose of vaccine, and the attenuated live vaccine was inoculated within 30 days；

6)Patients with the following diseases:

①Acute febrile diseases；

②Digestive system diseases (such as diarrhea, abdominal pain, vomiting, etc.) in the past 7 days；

③With congenital malformations or developmental disorders, genetic defects, severe malnutrition, etc；

④Congenital or acquired immune deficiency or autoimmune disease history or received immunomodulator treatment within 6 months, such as hormone; or monoclonal antibody; or thymosin; or interferon; but local medication (such as ointment, eye drops, inhalants or nasal spray) is allowed, and local medication should not exceed the dosage recommended in the

instruction manual or have any signs of systemic exposure；

⑤Chest CT examination was judged by researchers to be clinically significant or any positive for HBsAg, HCV antibody, HIV antibody or syphilis specific antibody；

⑥Neurologic disease or neurodevelopmental dysplasia (such as migraine, epilepsy, stroke, seizures in the last three years, encephalopathy, focal neurological deficit, guillain-barre syndrome, encephalomyelitis or transverse myelitis); history of mental illness or family history；

⑦Functional asplenia and splenectomy due to any reason；

⑧Patients with severe chronic diseases or disease in progress can not be smoothly controlled, such as diabetes, thyroid disease;

⑨Severe liver and kidney diseases; respiratory diseases that currently require routine drug treatment (e.g., chronic obstructive pulmonary disease [COPD], asthma) or any treatment that has aggravated respiratory diseases (e.g., exacerbation of asthma) in the last five years; history of severe cardiovascular diseases (such as congestive heart failure, cardiomyopathy, ischemic heart disease, arrhythmia, conduction block, myocardial infarction) Pulmonary heart disease) or myocarditis or pericarditis；

⑩Thrombocytopenia, coagulation dysfunction or anticoagulant therapy, etc；

⑪Cancer patients；

⑫Have received blood or blood related products within 3 months, including immunoglobulin; or have planned to use them during the study period；

7)Lactation women or pregnant women (including positive urine pregnancy test)；

8)Use any research or unregistered products (drug, vaccine, biological product or device) other than the research product in this study within 3 months, or plan to use them during the study period；

9)Dizzy with blood or needles；

10)The investigators believe that the presence of any disease or condition in the subjects may put them at an unacceptable risk; the subjects cannot meet the requirements of the

protocol; and the evaluation of vaccine response is interfered with.

9.3QUIT CRITERIA

1) AE or concomitant conditions that can not continue the test are found;

2) The subjects were unwilling to continue the clinical trial and asked to withdraw;

3) Participate in other clinical trials before the end of this clinical trial;

4) Other situations in which the investigator considered that the subjects were not suitable to continue to participate in the clinical study.

Regardless of the reason, the original record form should be kept for the subjects who quit the trial, and the last test results should be transferred to the final result, and the adverse reactions were analyzed with full data.

Subjects can stop participating in the trial at any time for any reason. The investigators should inform the subjects that they have the right to withdraw from the study at any time, and the withdrawn subjects will not be replaced. The investigator must distinguish the subjects who dropped out because of AE from those who dropped out for other reasons. According to the withdrawal time and situation of the subjects, the investigators determined the relevant safety inspection (for example, the subjects should withdraw from the test within 7 days after vaccination, at least the body temperature, blood biochemistry, blood routine and urine routine examination should be conducted; when the subjects quit the test within 7-30 days after vaccination, at least the body temperature should be measured; if the relevant examination could not be conducted due to the reasons of the subjects, it should be recorded in the original medical record ）.

investigators should try their best to contact the subjects who fail to return to the follow-up within the prescribed time. After the subjects withdraw from the study, the investigators should provide necessary guidance for the clinical conditions related to the trial, and follow up the AE / SAE to the outcome.

All data collected prior to the date of withdrawal / last contact with the subject was used for analysis. ECRF should record information related to the withdrawal of the study, such as: it was the subject / investigator who made the withdrawal decision and the possible reasons for the withdrawal (according to the quit criteria).

9.4 CONTRAINDICATIONS OF VACCINATION (FOLLOW-UP DOSE)

9.4.1 CONTRAINDICATIONS OF SUSPENSION OF VACCINATION

If any of the following occurs, the researchers will delay vaccination until the condition is relieved. The delay of vaccination should be within the specified time window as far as possible (see follow-up visitfollow-up visit).

- Fever (axillary temperature ≥ 37.3 ℃ on the day of enrollment of all the population during the follow-up);
- acute disease or acute attack of chronic disease (within 3 days before vaccination)
- The interval between other vaccines is insufficient (including the permitted vaccine before the trial vaccine);
- other contraindications for suspension of vaccination as determined by the investigators.

If the vaccination delay exceeds the time interval allowed by the study, it is necessary for the sponsor and the principal investigator to further discuss and determine whether to exclude the subject from the protocol analysis set. However, the subject will continue to participate in the study and remain in the full analysis set.

9.4.2 ABSOLUTE CONTRAINDICATION

If any of the following occurs, the investigator will terminate the vaccination of the subject.

1) The newly discovered or newly occurred cases meeting the first exclusion criteria before vaccination (except for items 1 and 2 of Article 5 and 6);

2) Severe or severe reaction after vaccination (Research vaccine or other vaccines caused by accident);

3) High fever occurred within 48 hours after inoculation (axillary temperature > 39.5 ° C);

4) Other serious adverse events: decide whether to terminate the study vaccine according to the treatment needs;

5) The investigators assessed any other reasons for discontinuation of the study vaccine.

9.5 COMPLETION AND QUIT THE STUDY

9.5.1 COMPLETION

Subjects who completed all visits in accordance with the protocol were considered to have completed the study.

9.5.2 QUIT THE STUDY

See 9.3 quit criteria for details.

9.5.3 TREATMENT OF LOST CONNECTION

When a subject fails to return on time for a follow-up examination, the researcher should make efforts to contact, confirm or recall the subject, or at least determine the health status of the subject, and record the efforts (such as telephone and SMS records) on the premise of fully respecting the rights of the subject.

9.5.4 DISCONTINUATION OF THE VACCINE

The subjects who stopped using the study vaccine were those who failed to receive the full study vaccine. The subjects who stopped the study vaccine did not necessarily withdraw from the study. According to the specific situation, the researchers could arrange them to continue to complete other research procedures or visits (such as safety or immunogenicity) according to the requirements of the protocol.

9.6 PROTOCOL DEVIATION / VIOLATION

Deviation from protocol: refers to any behavior that changes or does not follow the design or process of clinical trial protocol and is not approved by the ethics committee. The behavior that does not affect the rights, interests, safety and benefits of the subjects, or the integrity, accuracy and reliability of the test data as well as the evaluation of the safety or main indicators belong to the protocol deviation; those that affect the rights, interests, safety and benefits of the subjects, or the integrity, accuracy and reliability of the test data and the evaluation of the safety or main indicators are serious protocol deviations (protocol violation).

For the deviation / violation of the protocol during the research process, the on-site researchers shall report the fact, process, causes and impact of the incident to the research responsible institution. The principal investigators shall give their opinions on the handling of the incident, and the serious deviation / violation of the scheme shall be reported to the ethics Committee.

The investigator should carry out targeted training on the related links of the violation of the protocol and the staff concerned, so as to prevent the recurrence of similar events, and record the training process.

9.7 PREGNANCY EVENTS

Pregnancy is the exclusion condition of each dose of vaccination, and requires the subjects to take effective contraceptive measures from signing informed consent to the whole immunization within one year, but the subjects may still have accidental pregnancy in the process of participation. The pregnancy events occurred during the period from vaccination to the whole study period should be reported, and the researchers should fill in the "pregnancy event report form".

The researchers closely followed pregnant subjects or subject partners to obtain information about pregnancy outcomes (e.g., details of delivery and newborn conditions or termination of pregnancy), and updated the pregnancy event report form. The condition of the newborn was followed up for 1 year, and the follow-up was decided according to the non clinical results and 1-year observation results.

Pregnancy itself is not considered an SAE, but any complications during pregnancy will be considered as AE and in some cases can be considered as SAE, such as spontaneous abortion, stillbirth, stillbirth and congenital abnormalities of infants. In the absence of any abnormalities in the fetus, abortion due to the mother's personal decision is not considered as AE.

The treatment of pregnancy events during vaccination is as follows:

- If the pregnancy occurs after the first dose of vaccination, but the whole vaccination has not been completed, the female subjects are not allowed to participate in the follow-up visit,the investigators will contact the subjects regularly for pregnancy evaluation until

the end of pregnancy (neonatal follow-up for 1 year, abortion, abortion, etc.).

- If pregnancy is found after the subject has completed the whole course of vaccination, the subject can complete the study visit according to the trial scheme and the decision of the investigator.

9.8 SUSPENSION / TERMINATION OF TRIAL

New data on the vaccine for this study or any other study have been obtained, and suspension / termination of the trial is recommended by the administrative authority, the sponsor, the researcher, and / or the IRB;

Suspension criteria:

In case of any of the following conditions in any cohort, the trail shall be suspended and immediately reported to the ethics committee, the relevant departments of the provincial and State Drug Administration, and DSMB expert meeting shall be held for safety demonstration analysis to determine whether to continue the trail.

Events that cause a pause	**Number of cases /%**
Vaccine related deaths or serious life-threatening adverse events occurred during the study	≥1 case
Adverse events ≥ grade 3 and lasting for 48 hours after any dose of vaccination; and / or abnormal laboratory indicators with level ≥ 3 after any dose of vaccination	>15% of vaccinated population

Termination criteria:

In case of the following conditions in any cohort, the trial should be terminated and immediately reported to DSMB, ethics committee, provincial and State Drug Administration.

Events leading to termination	**Number of cases /%**
Adverse events ≥grade 3 and lasting for 48 hours after any	>30% of

dose of vaccination; and / or abnormal laboratory indicators with level ≥ 3 after any dose of vaccination	vaccinated population

If the study is terminated or suspended in advance, the sponsor will immediately inform the investigator, the ethics committee and the drug regulatory authorities of the reasons for the suspension or termination in accordance with the requirements of the corresponding registration regulations.

Regardless of the reason why the study was terminated in advance, the researcher should immediately inform the subjects and ensure that the subjects were properly followed up.

10.Experimental vaccine andconcomitant medication

10.1 EXPERIMENTAL VACCINE(LOW DOSE)

common name: Recombinant novel coronavirus vaccine (CHO cell)

production unit: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.

Batch number: see quality inspection report

Specification: 25 μ g / 0.5 ml / bottle

Active ingredients: recombinant ncp-rbd protein 25 μ g, aluminum hydroxide adjuvant 0.25 mg

Others: see quality inspection report

Testing unit: China Academy of food and drug control

Inspection Report No.: see quality inspection report

Term of validity: tentative 2 years

If the vaccine batches used in the trial are inconsistent with those recorded in the protocol, the responsible agency shall explain and record to the Ethics Committee (or according to the requirements of IRB) before the start of clinical trial.

10.2 EXPERIMENTAL VACCINE (HIGH DOSE)

common name: Recombinant novel coronavirus vaccine (CHO cell)

production unit: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.

Batch number: see quality inspection report

Specification: 25 μ g / 0.5 ml / bottle

Active ingredients: recombinant ncp-rbd protein 25 μ g, aluminum hydroxide adjuvant 0.25 mg

Others: see quality inspection report

Testing unit: China Academy of food and drug control

Inspection Report No.: see quality inspection report

Term of validity: tentative 2 years

If the vaccine batches used in the trial are inconsistent with those recorded in the protocol, the responsible agency shall explain and record to the Ethics Committee (or according to the requirements of IRB) before the start of clinical trial.

10.3 PLACEBO

common name: Recombinant novel coronavirus vaccine (CHO cell)

production unit: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.

Batch number: see quality inspection report

Specification: 0.5ml/bottle

Active ingredient: no antigen, 0.25mg aluminum hydroxide adjuvant

Others: see quality inspection report

Testing unit: National Institutes for Food and Drug Control

Inspection Report No.: see quality inspection report

Term of validity: tentative 2 years

10.4 PACKAGING AND LABELING

The outer package and inner package of the research vaccine were pasted with the same number label, and a pre printed self-adhesive label with the same number was placed in the packaging box to paste the research medical records of the subjects. The label contains the following information:

1) Large package label of vaccine: product name, number range, storage conditions, enterprise name, batch number and expiration date. Words like "for clinical research

only" should be printed on the large package box.

2) Package label of single vaccine: product name, study number, vaccine specification and expiration date, vaccine batch number, dosage, subject's abbreviation, and words like "for clinical research only".

3) Vaccine bottle label: vaccine number.

4) Built in label (used to paste research medical records): vaccine number, subject abbreviation.

After the use of the vaccine, the subject's initials should be filled in the outer package label and the built-in label (used to paste on the research medical record) of the single vaccine, and the vaccination personnel should vaccinate the vaccine with the corresponding number after verification.

10.5 VACCINE STORAGE AND TRANSPORTATION

The vaccine should be stored and transported away from light at 2-8 ℃, and freezing is strictly prohibited. It is necessary to monitor and record the storage temperature every day (under the premise of automatic temperature monitoring alarm, holidays can be arranged according to the specific situation of the site). If the storage and transportation conditions are beyond the specified scope, the on-site researchers shall immediately contact the clinical responsible unit personnel and the sponsor, and shall not use the research vaccine until the sponsor's opinions are obtained.

10.6 VACCINE USAGE

Before using, it should be fully shaken and observed with naked eyes whether there are particles. In case of unshakable clots and foreign matters, they should not be used.

10.6.1 INOCULATION SITE AND WAY

The subjects are inoculated with 3 doses of vaccine at an interval of 30 days. Intramuscular injection of deltoid muscle of upper arm.

10.6.2 INOCULATION DOSE

The experimental vaccine and placebo were 0.5ml per person each time.

10.7 REMINDERS AND PRECAUTIONS

Like all vaccines, the vaccination site should be equipped with appropriate emergency treatment measures and equipped with epinephrine and other drugs for use in case of occasional severe allergic reaction after vaccination. After vaccination, patients should be observed for 30 minutes and continue to observe for 1.0 hours before leaving the hospital with the permission of the investigator.

10.8 RESERVE VACCINE

If the test vaccine / placebo is found to be damaged during the test, the investigator shall log in the reserve vaccine acquisition system to obtain the corresponding number of reserve vaccine (when the number of spare vaccine is insufficient, it can be added at any time).

10.9 CONCOMITANT MEDICATION

At each visit / contact, the investigator should ask the subjects whether they have taken any drugs and received any vaccines. All drug combinations / vaccines (except vitamins and / or food additives) should be recorded. Researchers should transcribe the drug combination to eCRF.

Concomitant medication: All drugs except the research vaccine used by the subjects within 30 days after the subjects signed the informed consent form to receive the last dose of vaccine, and all drugs except the research vaccine used by the subjects due to SAE and pregnancy within 30 days after the last dose of vaccine and within 1 year, including antibiotics, antiviral drugs, antipyretic analgesics, anti allergic drugs, biological products (vaccines), Chinese (patent) drugs, etc（ Vitamins and / or food additives).

According to the following 10 categories, the data management personnel classified the combined drugs according to the following 10 categories:

(1) Hormone / steroid drugs and other immunosuppressants;

(2) Anti allergic drugs;

(3) Antipyretic / analgesics / NSAIDs;

(4) Vaccines and biological products;

(5) Immunoglobulin and other blood products;

(6) antibiotic;

(7) Antiviral drugs;

(8) Chinese patent medicine;

(9) Traditional Chinese medicine prescription;

(10) Others.

Permitted vaccines: The use of vaccines should follow the inclusion / exclusion criteria. Emergency vaccination, such as rabies or tetanus, should not be restricted. However, the use of vaccines should be truthfully recorded according to the requirements. Other vaccines should be inoculated before the vaccination of research vaccine. The interval between recombinant subunit vaccine and inactivated vaccine should be at least 14 days. The interval between live attenuated vaccine and research vaccine should be at least 30 days. After inoculation of other vaccines after vaccination of research vaccine, the interval between inactivated vaccine and attenuated live vaccine is only 7 days

Permitted drugs: During the trial period, if the subject has AE, he should be allowed for necessary drug treatment, and record the medication information according to the requirements. The use of contraceptives should also be allowed due to the contraceptive requirements of the subjects in this trial; however, the medication information of any drug should be recorded according to the requirements.

Contraindications: Preventive drugs refer to the drugs given when there are no symptoms and expected vaccination reactions (for example, when there is no fever, antipyretic drugs are taken to prevent the occurrence of fever, and then antipyretic drugs are regarded as preventive drugs). At the time of enrollment, the subjects should be asked about the drugs in use to confirm that the subjects did not use preventive drugs, including antipyretic analgesic and antiallergic drugs.

The following drugs / preparations need to be collected throughout the study period, and the use of the following drugs may affect subjects' access to the PPS (protocol compliance data set) analysis set:

- Use of any research or unregistered product (drug, vaccine) other than the research vaccine in this study during the study period;

- Use of any research vaccine or similar vaccine during the study;
- Long term use (≥ 14 days) of immunosuppressants or other immunomodulatory drugs (such as glucocorticoids);
- Immunoglobulin and other immunopotentiators were injected after the first dose of vaccination.

During data cleaning, the data manager will establish a detailed and comprehensive list of reasons to be removed from the PPS analysis set of basic immunization, and make decisions on whether to include the reasons in the PPS analysis when reviewing the decision.

11. Trial process

Figure 2 visit follow-up visit

Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**P1**	**V9**	**V10**	**V11**	**P2**	**V12**	**P3**	**V13**	**V14**
Time(days)	D-7	D0	V2+4	V2+7	V2+14	V2+30	V6+4	V6+7	V6+14	V6+30	V9+4	V9+7	V9+14	V9+30	V9+90	V9+180	V9+365
Window period (days)	-7~0	NA	±1	+1	±1	+7	±1	+1	±1	+10	±1	+1	±1	+7	±30	+30	+30
informed consent	●																
Demographic information	●																
Height and weight	●																
medical history	●	●				●				●							
Armpit temperature	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●
Vital signs	●	●	●	●		●	●	●		●	●	●					
physical examination	●	●	●			●	●			●	●						
Blood routine, urine routine, blood biochemistry	●	●	●			●	●			●	●						
Blood pregnancy test	●																
Urine pregnancy test		●				●				●							
Virus serological examination	●																
PT APTT TT FIB	●																

Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**P1**	**V9**	**V10**	**V11**	**P2**	**V12**	**P3**	**V13**	**V14**
Time(days)	D-7	D0	V2+4	V2+7	V2+14	V2+30	V6+4	V6+7	V6+14	V6+30	V9+4	V9+7	V9+14	V9+30	V9+90	V9+180	V9+365
Window period (days)	-7~0	NA	±1	+1	±1	+7	±1	+1	±1	+10	±1	+1	±1	+7	±30	+30	+30
12 lead ECG	●																
Chest CT	●																
Screening of IgM and IgG antibodies against SARS-CoV-2	●																
Real time RT-PCR nucleic acid screening for SARS-CoV-2	●	●				●				●							●
Audit inclusion & exclusion criteria	●	●															
Contraindications to vaccination						●				●							
Assign random number		●															
Humoral immunogenicity blood sampling (about 8ml)		●			●	●		●		●		●		●		●	
Cellular immunogenicity blood sampling (about 30ml)		●			●	●		●		●		●		●		●	
vaccination		●				●				●							
30 minutes after inoculation		●				●				●							
Issue and train diary cards, thermometer and ruler		●				●				●							
Recycle diary card				●				●				●					
Issue and training contact card				●				●				●					
Recycle contact card						●				●				●			
Recording adverse events	●	●	●	●	●	●	●	●	●	●	●	●	●	●			
Reporting serious adverse events	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Concomitant medication	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Note: during the screening period and before each dose of vaccination, only routine blood and urine biochemical test results were accepted within 7 days before the test. Blood volume of blood routine is about 2.0-4.0ml; Blood volume of blood biochemistry is about 3.0-4.0ml; Blood volume of blood pregnancy is about 2.0-4.0ml; blood volume of virus serology and coagulation is about 4.0-8.0ml.

P1,P2, P3 were telephone visits, the rest were hospital visits.

Follow-up visit Description:

V1：D -7～0

The subjects whose basic information met the requirements of the experiment were recruited for screening. The subjects must sign the informed consent form in writing on a voluntary basis before participating in the screening. Screening physical examination was carried out within 7 days before vaccination. Subjects who met all the inclusion criteria and did not meet the exclusion criteria could be enrolled. Adverse events and concomitant medication after signing the informed consent form should be recorded truthfully and reported by SAE.

The checks to be completed for screening include:

General information: demographic information (including but not limited to name, gender, age, nationality, place of residence, work and life history of epidemic area, etc.); height and weight (height and weight were measured after taking off coat and shoes); medical history inquiry (the researcher consulted and recorded the subjects' previous diagnosis of acute and chronic medical conditions, operation conditions, drug and food allergy history, etc.); Measure axillary temperature.

Physical examination and laboratory examination: vital signs assessment (blood pressure, pulse); physical examination (skin and mucous membrane, lymph node, head, neck, chest, abdomen, spine / limbs, the same as the following physical examinations); blood routine, blood biochemistry and urine routine; blood pregnancy (only for women); virus serological examination (human immunodeficiency virus (HIV) antibody, hepatitis B surface antigen, hepatitis B surface antigen, etc.) Hepatitis C antibody, syphilis specific antibody, referred to as virus serological examination in this program, and four coagulation items (prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen); 12 lead electrocardiogram; chest imaging; SARS-CoV-2 IgM and LGG antibody screening and real-time fluorescent RT-PCR nucleic acid screening for SARS-CoV-2 (patients without previous history of coronavirus infection or onset, those without contact history should be screened for SARS-CoV-2, and those with positive results could be excluded).

The investigators conducted the inclusion and exclusion criteria and vaccination contraindications audit, and screened out qualified subjects.

V2：D 0

The investigator will inform the eligible subjects to arrive at the experimental center, and they will inquire about their medical history, measure their axillary temperature and vital signs, physical examination, blood routine, urine routine, blood biochemistry, urine pregnancy examination (only for women), and conduct real-time fluorescent RT-PCR nucleic acid screening for SARS-CoV-2 (those without previous coronavirus infection or disease history and those without contact history will be screened for SARS-CoV-

2. Any subjects with positive results may be excluded). If the subjects were screened and qualified on the same day, the above examination could not be repeated.

According to all the results of screening examination and D0 results, the investigators judged the eligibility of the subjects, and the qualified candidates were assigned random numbers. Before vaccination, humoral immunogenic blood and cellular immunogenic blood were collected (if no special instructions, 8ml of humoral immunogenicity and 30ml of cellular immunogenicity) were collected before vaccination, and then the first dose of vaccine was inoculated. Close observation was made for 30 minutes after inoculation.

The subjects were kept for observation for at least 1.0 h and could leave the hospital with the consent of the investigators. Before leaving the hospital, the investigator trained the subjects to fill in the diary card, and issued ruler and thermometer to measure the body temperature and injection site reaction. The subjects filled in the diary card by themselves. In case of any abnormality, they should contact the researcher in time and fill in the diary card. The investigators timely handled the adverse events within 7 days after the vaccination, filled in the original medical records, and reported the serious cases.

V3：V2+4

After the subjects returned to the hospital, the investigator checked the diary card, and carried out axillary temperature and vital signs measurement, physical examination, blood routine examination, blood biochemistry and urine routine examination, and the subjects could leave the hospital with the permission of the researcher. After discharge, the subjects monitored their body temperature every day and filled in the diary card. In case of any abnormality, it is necessary to contact the investigator in time.

V4：V2+7

After the subjects returned to the hospital, the investigator checked the recycled diary card, measured the axillary temperature and vital signs of the subjects, issued the contact card, and trained the subjects to fill in the contact card, measure the body temperature and injection site reaction (if any), and the subjects can leave the hospital with the permission of the investigator. After discharge, the subjects monitored their body temperature every day and filled in the contact card. In case of any abnormal situation, it is necessary to contact the investigator in time and fill in the contact card.

Note: subjects in the second stage (high-dose: placebo group) enter the group after the subjects in the first stage (low-dose: placebo group) were vaccinated for 7 days,and DSMB evaluated the safety results and agreed. In the second phase (high-dose: placebo group), the safety data of subjects after the first dose of vaccination for 7 days should also be submitted to DSMB for evaluation.

V5：V2+14

The investigators measured the axillary body temperature of the subjects, and collected the humoral and cellular immunogenic blood samples. Check the contact card.

V6：V2+30

The contact card will be collected and checked by the investigator. The medical history inquiry, underarm temperature and vital signs measurement, physical examination, blood routine examination, blood biochemistry, urine routine examination, urine pregnancy test (only for women) and real-time fluorescent RT-PCR nucleic acid screening of SARS-CoV-2 are carried out. After reviewing the vaccination taboo, the investigator willjudge whether the subject is suitable for vaccination of another dose. If the second dose is suitable for vaccination, humoral immunogenicity blood collection and cellular immunogenicity blood collection should be conducted before vaccination; the second injection should be given to qualified subjects; if the subjects have abnormal conditions, vaccination can be delayed for 7 days, and immunogenic blood collection should be conducted before vaccination on the day of vaccination.

The patients were observed closely for 30 minutes after inoculation, and then they could leave the hospital after 1.0 hours of safety assessment and permission.

Before leaving the hospital, the investigator trained the subjects to fill in the diary card. During the period of leaving the hospital, the researcher should monitor the body temperature every day. If there is any abnormal situation, the investigator should contact the researcher in time and fill in the diary card.

The investigators timely handled the adverse events within 7 days after the vaccination, filled in the original medical records, and reported the serious cases.

V7: V6+4

After the subjects returned to the hospital, the researcher checked the diary card, and carried out axillary temperature and vital signs measurement, physical examination, blood routine examination, blood biochemistry and urine routine examination, and the subjects could leave the hospital with the permission of the investigator. After discharge, the subjects monitored their body temperature every day and filled in the diary card. In case of any abnormality, it is necessary to contact the investigator in time.

V8: V6+7

The investigator measured the axillary temperature and vital signs of the subjects, checked and recovered the diary cards, collected blood for cellular immunogenicity and humoral immunogenicity, and issued contact cards. The subjects could leave the hospital with the permission of the investigator. After discharge, the subjects monitored their body temperature every day and filled in the contact card. In case of any abnormal situation, it is necessary to contact the investigator in time and fill in the contact card.

Note: in the second stage (high dose: placebo group), DSMB needs to evaluate all safety results before this stage 7 days after completing the whole vaccination.

V9：V6+30

The contact card will be collected and checked by the investigator. The medical history inquiry, armpit temperature and vital signs measurement, physical examination, blood routine examination, blood biochemistry, urine routine examination, urine pregnancy test (only for women) and real-time fluorescent RT-PCR nucleic acid screening of SARS-CoV-2 will be carried out. After reviewing the vaccination taboo, the investigator will judge whether the subject is suitable for vaccination of another dose. If the subject is suitable for the third dose of vaccination, humoral immunogenicity blood collection and cellular immunogenicity blood collection should be conducted before vaccination; the third injection should be given to qualified subjects; if the subjects have abnormal conditions, vaccination can be delayed for 10 days, and immunogenic blood collection should be conducted before vaccination on the day of vaccination.

The subjects will be observed closely for 30 minutes after inoculation, and then they could leave the hospital after 1.0 hours of safety assessment by and permission from the investigator.

Before leaving the hospital, the investigator will train the subjects to fill in the diary card. During the period of leaving the hospital, the subjects should monitor the body temperature voluntarily every day, and should contact the investigator in time in case of any abnormal situation and fill in the diary card.

The investigators should handle in a timely manner the adverse events within 7 days after the vaccination, fill in the original medical records, and report the serious cases.

V10: V9+4

After the subjects return to the hospital, the investigator will check the diary card, and carry out armpit temperature and vital signs measurement, physical examination, blood routine examination, blood biochemistry, urine routine examination, and the subjects could leave the hospital upon permission from the investigator. After leaving the hospital, the subjects should monitor the body temperature voluntarily every day and fill in the diary card, and should contact the investigator in time in case of any abnormal situation.

V11: V9+7

The investigator will carry out armpit temperature and vital signs measurement, check the collected diary card, carry out cellular and humoral immunogenicity blood sampling, and distribute the contact card. The subjects could leave the hospital upon permission from the investigator. After leaving the hospital, the subjects should monitor the body temperature voluntarily every day and enter on the contact card, and should contact the investigator in time in case of any abnormal situation and fill in the contact card.

V12: V9+30

The investigator will collect and check contact card, carry out armpit temperature of the subjects, carry out cellular and humoral immunogenicity blood sampling.

V13：V9+180

The investigator measured the axillary temperature of the subjects, collected blood for cellular immunogenicity and humoral immunogenicity. At the same time, SAE and drug combination were recorded and reported to SAE.

V14：V9+365

Axillary temperature measurement and real-time fluorescent RT-PCR nucleic acid screening of SARS-CoV-2 were carried out. The occurrence and combination of SAE were recorded and reported to SAE.

P1, P2, P3:

Telephone visit. The investigator conduct a telephone visit for the subjects, ask about the recent situation, especially whether they have any suspected symptoms of new coronavirus infection. If the subjects have any discomfort, they should visit the hospital in time for treatment and record in the original medical record. Meanwhile, any SAEs and concomitant medication should be recorded. Additional visits may be made by the investigator according to the situation.

The subjects were informed of the following precautions:

Because the novel coronavirus novel coronavirus has not disappeared, the new type of coronavirus infection symptoms such as fever and respiratory symptoms should be immediately contacted. During the visit to the local hospital, we should guard against the risk of the new coronavirus exposure.

11.1 SUBJECT RECRUITMENT

After the trial site is determined and approved by the ethics committee, before the start of the study, the researcher or its authorized person will issue recruitment notice to the subjects who meet the requirements of age, vaccination history and health status, contact and register to participate in the study.

11.2 INFORMED CONSENT:

The informed consent process should be completed prior to any research procedure. Two copies of the ICF were kept by the participants before the study. After signing the informed consent, the researchers need to conduct epidemic safety training for the subjects.

11.3 SCREENING

According to the "inclusion and exclusion criteria", the investigator inquired about the medical history, residence, place of life, occupation and other information of the subjects, and carried out physical examination for all subjects, including height, weight, vital signs, physical examination (skin and mucosa, lymph nodes, head, neck, chest, abdomen, spine /

limbs), etc. Screening information and demographic data (such as date of birth, gender, and ethnicity) were included in the study medical record.

11.4 RANDOMIZATION

The eligible subjects were given random number according to the sequence. The random number is used to identify all procedures that occur after the subjects are randomized into groups. Once the random number was assigned to one subject, it could not be reassigned to other subjects; the number of dropped off subjects was not redistributed regardless of whether they were vaccinated or not. The investigators filled in the screening number and initials of the subjects in the randomization form, and filled in the vaccine number in the study medical record.

11.5 BIOLOGICAL SAMPLE COLLECTION

11.5.1 BLOOD SAMPLE COLLECTION

Blood biochemistry (alanine aminotransferase, aspartate aminotransferase, total bilirubin, urea, creatinine, fasting blood glucose), blood routine (white blood cell, platelet, hemoglobin, lymphocyte) and urine routine (urine egg white, urine red blood cell) were detected in the screening period, before and 4 days after each dose of inoculation. The test of blood biochemistry (creatine kinase and lactate dehydrogenase) was increased before and 4 days after the third dose of inoculation. Women of childbearing age should be tested by blood pregnancy test during screening period.

SARS-CoV-2 IgM and IgG antibodies were detected in all subjects during the screening period.

About 30 ml blood samples are collected from all subjects before each dose, 14 days after the first dose of inoculation, 7 days after the second dose of vaccination, 7 days after the third dose of vaccination, and 1 and 6 months after the whole course of vaccination for cellular immune test.

About 8 ml blood samples are collected from all subjects before each dose, 14 days after the first dose, 7 days after the second dose, 7 days after the third dose, and 1 month and 6 months after the whole vaccination for humoral immune test.

11.5.2 URINE SAMPLE COLLECTION (INCLUDING PREGNANCY TEST)

The urine routine (urine protein, urine red blood cell) was detected in the screening period, before and 4 days after each dose.

Women of childbearing age were examined by urine pregnancy test before each dose of vaccination.

11.5.3 OTHER SAMPLES

All subjects were sampled for real-time RT-PCR nucleic acid detection of SARS-CoV-2 in the screening period, before each dose of vaccination and 1 year after the whole vaccination.

11.6 VACCINATION

According to the assigned random number, the corresponding vaccine number was obtained. After checking the number on the study medical record, the subject's abbreviation was filled in the label of the outer package of the vaccine, and the built-in label of the vaccine was filled in and pasted on the designated position of the study medical record. The information of the subjects was checked again before vaccination. After shaking the vaccine well, 0.5ml vaccine was injected intramuscularly into the deltoid muscle of the upper arm.

11.6.1 FIRST DOSE OF INOCULATION

According to the random number, the subjects completed the first dose of vaccination on the day of enrollment.

11.6.2 SECOND DOSE INOCULATION

The second dose was inoculated at an interval of 30 days, and the inoculation window period was + 7 days. If the subjects have vaccination contraindications, please refer to section 9.4 to postpone or stop the study vaccination.

11.6.3 THIRD DOSE INOCULATION

The third dose will be inoculated at an interval of 30 days, and the inoculation window period is + 10 days. If the subjects have vaccination contraindications, please refer to section 9.4 to postpone or stop the study vaccination.

11.7 MEDICAL OBSERVATION

After each dose of the study vaccine, the subjects were observed closely for 30 minutes.

According to the visiting follow-up visit, the research doctors conducted relevant operations on the subjects, observed the reaction of the subjects, handled the adverse events in time, and filled in the original medical records and reported them in time.

Observe for 1.0 h after each dose of vaccination, and leave the hospital after the evaluation of the investigator. Fill in the diary card within 7 days, and fill in the contact card in 8-30 days. The contact card of the last dose should be collected and reviewed at the time of blood collection 30 days after the whole vaccination.

The safety was observed until one year after the whole vaccination.

The investigators emphasized the copy of the informed consent form and the investigator's telephone number on the contact card to instruct them to contact the investigator immediately in case of any signs, symptoms and events requiring hospitalization.

11.8 SAFETY FOLLOW-UP AND EVALUATION

11.8.1 DEFINITION OF ADVERSE EVENTS

Adverse events（AE）: Adverse medical events that occur after patients or clinical trial subjects receive a drug, but they are not necessarily related to the treatment.

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

Serious adverse event (SAE): in the process of clinical trial, the events such as hospitalization, prolonged hospitalization time, disability, impact on work ability, life-threatening or death, and congenital malformation occurred.

11.8.2 FOLLOW UP TIME AND METHOD

- ✓Close observation was conducted for 30 minutes after each dose of vaccine, and the immediate AE was observed;
- ✓The research doctors observed whether there were any adverse events during the hospitalization, and handled and filled in the original records in time;
- ✓The subjects were allowed to leave hospital without safety problems during their stay in hospital. Meanwhile, diary cards were issued to the subjects to record the AE from each dose to 7 days

after inoculation;

- ✓The subjects returned to the hospital 7 days after each dose of vaccination, and the researcher checked the information of diary card and issued contact card to record the AE of 7-30 days after each dose of vaccination;
- ✓From the first dose of the vaccine to one year after the whole vaccination, SAE was collected by the way of active reporting by the subjects and regular telephone follow-up by investifators.
- ✓During each visit, the investifator emphasized that the subject could contact the researcher at any time through the telephone number on the contact card / copy of informed consent form.

11.8.3 FOLLOW-UP CONTENT

The safety observation included all the solicitation AE, non solicitation AE, and AE and SAE related to or unrelated to vaccination during the clinical trial.

11.8.3.1 SOLICITATION ADVERSE EVENTS

Solicitation adverse events：The following AE occurred within 7 days after vaccination of the study vaccine:

- ✓Reaction of inoculation site(local): pain, swelling, induration, redness, rash, pruritus, cellulitis;
- ✓Non inoculation site (systemic) reactions: fever, cough, dyspnea, diarrhea, anorexia, nausea, vomiting, muscle pain (non vaccination site), arthritis, joint pain, headache, fatigue, fatigue, acute allergic reaction, irritation or inhibition, mental disorder

11.8.3.2 NON SOLICITATION ADVERSE EVENTS

Any AE other than solicitation AE or solicitation AE occurring outside the solicitation period.

11.8.3.3 ABNORMAL LABORATORY EXAMINATION

If there are abnormal items in 8.1.3 of the protocol after vaccination, the abnormal items should be retested and followed up closely.

Compared with the baseline specified in the protocol, if the laboratory examination index is abnormal, it should be collected as AE. If there are clinical symptoms or clinical

significance, they should be recorded as AE; if certain medical measures are taken for the treatment of AE, the medical measures taken (such as start and end time, etc.) should be recorded at the same time of recording AE; if there are therapeutic drugs, it is also necessary to fill in the form and combined medication table.

11.8.3.4 SERIOUS ADVERSE EVENTS

Serious adverse event (SAE) refers to a medical event that meets any of the following conditions:

a) Leading to death

b) Life threatening;

c) Carcinogenesis, teratogenesis and birth defects;

d) Lead to disability or organ function damage, affect work ability;

e) Causing hospitalization or prolonging hospitalization time;

f) Lead to other important medical events, if not treated, the above-mentioned situations may occur.

11.8.4 SAFETY ASSESSMENT

11.8.4.1 SEVERITY OF ADVERSE EVENTS

According to NMPA's "*guiding principles for classification of adverse events in clinical trials of preventive vaccines (Revised Version)"*, the severity of local and systemic reactions after vaccination was determined. According to the characteristics of subjects in this trial, the indicators were adjusted accordingly. The severity grading standards of adverse events were as follows:

Table 4 severity classification of adverse events at vaccination site (local)

Symptoms / signs	**Level 1**	**Level 2**	**Level 3**	**Level 4**
Rash *, erythema#**				
＞14 y	The diameter is 2.5 ～ < 5 cm or the area is 6.25 ～ < 25 cm^2, which does not affect or slightly affect daily	Diameter 5 ～ < 10 cm or area 25 ～ < 100 cm^2 or affect daily life	Diameter ≥ 10 cm or area ≥ 100 cm^2 or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or serious impact on daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis

	life			
Induration*, swelling#**				
＞14 y	The diameter is 2.5 ～ < 5 cm or the area is 6.25 ～ < 25 cm^2, which does not affect or slightly affect daily life	Diameter 5 ～ < 10 cm or area 25 ～ < 100 cm^2 or affect daily life	Diameter ≥ 10 cm or area ≥ 100 cm^2 or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or serious impact on daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Pain / tenderness(optional; for subjects unable to express pain autonomously, tenderness was used)				
Pain	Does not affect or slightly affects physical activity	Affect physical activity	Affect daily life	Loss of basic self-care ability, or hospitalization
tenderness	To resist or withdraw from contact or touch	Crying after touching or touching, but can be soothed	Constant crying can't be comforted	Need emergency or hospitalization
Others				
pruritus	The pruritus at the inoculation site was relieved by itself or within 48 hours after treatment	The pruritus at the inoculation site did not relieve within 48 hours after treatment	Affect daily life	NA
Cellulitis	NA	Non injectable therapy (e.g. oral antibacterials, antifungal, antiviral drugs) is required	Need intravenous therapy (such as intravenous antibacterials, antifungal, antiviral drugs)	Sepsis, or tissue necrosis

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

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

#For induration, swelling, rash and erythema, and the evaluation and grading should be based on the functional grade and the actual measurement results, and the index with higher grade should be selected.

Table 5 severity classification of adverse events at non vaccinated sites (systemic)

Organ system symptoms / signs	**Level 1**	**Level 2**	**Level 3**	**Level 4**

Fever [&][axillary temperature (℃)]				
＞14 y	37.3～＜38.0	38.0～＜38.5	38.5～＜39.5	≥39.5 for more than 3 days
Gastrointestinal system				
diarrhea	Mild or transient, 3-4 times / day, abnormal stool characteristics, or mild diarrhea lasting less than 1 week	Moderate or persistent, 5-7 times / day, abnormal stool characteristics, or diarrhea > 1 week	More than 7 times / day, abnormal stool characteristics, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, intravenous infusion ＞ 2L	Hypotension shock, requiring hospitalization
anorexia	Loss of appetite, but no reduction in food intake	Appetite and food intake decreased, but body weight did not decrease significantly	Loss of appetite and weight	Need intervention (e.g. gastric tube feeding, parenteral nutrition)
vomit	1-2 times / 24 hours without affecting activities	3-5 times / 24 hours or limited activity	More than 6 times in 24 hours or intravenous rehydration is required	Shock due to hypotension requires hospitalization or other nutrition
nausea	Transient (< 24 hours) or intermittent and food intake is basically normal	Persistent nausea leads to reduced food intake (24-48 hours)	Persistent nausea results in almost no food intake (> 48 hours) or the need for intravenous rehydration	Life threatening (e.g. hypotension shock)
Musculoskeletal and connective tissue				
Muscle pain (non inoculated site)	Does not affect daily activities	Slightly affect daily activities	Severe muscle pain, seriously affecting daily activities	Emergency or hospitalization
arthritis	Mild pain with inflammation, erythema, or joint swelling; but does not interfere with function	Moderate pain with inflammation, erythema or joint swelling; impairs function but does not affect daily activities	Severe pain with inflammation, erythema or joint swelling; affects daily activities	Permanent and / or disabled joint injury
Arthralgia	Mild pain, does not interfere with function	Moderate pain; requires analgesics and / or pain impairs function but does not affect daily activities 报错 笔记	Severe pain; need for analgesics and / or pain affecting daily activities	Disability pain

nervous system				
headache	It does not affect daily activities and does not require treatment	Transient, slightly affect daily activities, may need treatment or intervention	It seriously affects daily activities and needs treatment or intervention	Intractable, need emergency or hospitalization
respiratory system				
cough	Transient, without treatment	Continuous cough, effective treatment	Paroxysmal cough, uncontrollable treatment	Emergency or hospitalization
dyspnea	Dyspnea during exercise	Dyspnea due to normal activities	Dyspnea at rest	Dyspnea, need oxygen therapy, hospitalization or assisted breathing
psychic system				
To provoke or restrain	Mild irritation or mild inhibition	Irritable or sleepy	Unable to soothe or react poorly	NA
Mental disorders (including anxiety, depression, mania, and insanity) should be reported in detail	Mild symptoms, no need to see a doctor or behavior does not affect or slightly affect daily life	Have clinical symptoms, need to see a doctor or behavior affect daily life	Need to be hospitalized or unable to support daily life	There is a tendency to hurt oneself or others, or acute mental disorder or loss of basic self-care ability
immune system				
Acute allergic reaction**	Local urticaria (blister) without treatment	ia, need treatment or mild vascular edema, no treatment	Extensive urticaria or vascular edema requires treatment or mild bronchospasm	Anaphylactic shock or life-threatening bronchospasm or laryngeal edema
Others				
Fatigue	Does not affect daily activities	Affect normal daily activities	Seriously affect daily activities, unable to work	Emergency or hospitalization

Note: *The axillary temperature is usually adopted in China, and it is converted into oral temperature and rectal temperature when necessary. Usually, oral temperature = axillary temperature + 0.2℃; rectal temperature = axillary temperature + (0.3~0.5℃). When persistent high fever occurs, the cause of the high fever should be identified as soon as possible.

**Refers to type I hypersensitivity.

Table 6 classification of blood biochemical indexes

Detection index		Level 1	Level 2	Level 3	Level 4
liver function (ALT, AST increased)		1.25 ～ ＜ 2.5 ×ULN	2.5～＜5.0×ULN	5.0～＜10×ULN	≥10×ULN
Total bilirubin increased (mg / dl; μ mol / L)		1.1～＜1.6×ULN	1.6～＜2.6×ULN	2.6～5.0×ULN	≥5.0×ULN
Creatinine (creatinine increased)*		1.1~1.3 ×ULN	3-1.8 × ULN or 1.3-1.5 times higher than the baseline of subjects	1.8-3.5 × ULN or 1.5-2.0 times higher than the baseline of subjects	≥3.5 ×ULN or 2.0 times higher than baseline
Hyperglycemia (Glu, mmol / L)	Fasting	6.11～＜6.95	6.95～＜13.89	13.89～＜27.75	≥27.75
	Non fasting	6.44～＜8.89	8.89～＜13.89	13.89～＜27.75	≥27.75
Hypoglycemia (Glu, mmol / L)		3.05～＜3.55	2.22～＜3.05	1.67～＜2.22	＜1.67
creatine kinase		1.25 ～ ＜ 1.5 ×ULN	1.5 ～ ＜ 3.0 ×ULN	3.0～＜10 ×ULN	≥10×ULN

Note: ULN refers to the upper limit of the normal range.

表 7 血液常规检查分级表

Table 7 blood routine examination grading table

Test index / classification	Level 1	Level 2	Level 3	Level 4
Leukocyte elevation (WBC, 109 / L)	11~＜13	13～＜15	15～＜30	≥30
Leucopenia (WBC, 109 / L)	2.000～2.499	1.500～1.999	1.000～1.499	＜1.000
Lymphocyte decrease (ly, 109 / L)	0.75～1.00	0.5～0.749	0.25～0.49	＜0.25
Thrombocytopenia (PLT, 109 / L)				
＞12y	125～140	100～124	25～99	＜25
Low hemoglobin (g / dl)				
Male ≥ 13 years old	10.0～10.9	9.0～＜10.0	7.0～＜9.0	＜7.0
Female ≥ 13 years old	9.5～10.4	8.5～＜9.5	6.5～＜8.5	＜6.5

Table 8 grading table of routine urine examination

Test index	Level 1	Level 2	Level 3	Level 4
Urine protein (pro) (urine test paper)	1+	2+	3 + or higher	NA
Red blood cells (microscopic examination) [RBC / HPF per high power field (excluding female menstrual period)]	6～＜10	≥10	Gross hematuria, with or without blood clots; or with or without red blood cells; or requiring treatment	Emergency or hospitalization

Table 9 classification of other adverse events

Level 1	Level 2	Level 3	Level 4	Level 5

Mild: short time (< 48h) or slight discomfort, no effect on activity, no need for treatment	Moderate: mild or moderate activity restriction, may require medical treatment, no or only mild treatment	Severe: the activity is obviously limited and needs to be treated and hospitalized	Critical: may be life-threatening, seriously restricted activities, need to be monitored and treated	Death

11.8.4.2CORRELATION WITH THE VACCINE UNDER STUDY

For the expected or unexpected AE (solicitation or non solicitation AE), the invesgator should take measures to judge the correlation with vaccination in time, timely discover the SAE related to vaccination and mass and tendentious AE during the clinical trial, and timely suspend and terminate the clinical trial, so as to minimize the harm to the subjects.

The general principles of relevance judgment are as follows**:**

✓Definitely related :There is evidence of vaccination of research vaccine; the occurrence of adverse events and the time sequence of vaccination are reasonable; the occurrence of adverse events is explained by research vaccine more reasonably than other reasons; repeated vaccination of research vaccine is positive; adverse event situation is consistent with previous knowledge of this or this kind of vaccine.

✓Likely to be related : there is evidence of vaccination of research vaccine; the occurrence of adverse events and the timing of vaccination are reasonable; the occurrence of adverse events is explained by research vaccine more reasonable than other reasons.

✓Possibly related: there is evidence of vaccination of research vaccine; the occurrence of adverse events and the time sequence of vaccination are reasonable; the occurrence of adverse events can not be ruled out to be caused by the research vaccine, but also may be caused by other reasons

✓Possibly unrelated: there is evidence of vaccination research vaccine; adverse events are more likely to be caused by other reasons; repeated vaccination test is negative or uncertain.

✓Definitely unrelated: subjects did not use the research vaccine; or the occurrence of adverse events was illogical with the time sequence of vaccination; or there were other significant reasons that could lead to adverse events.

✓In statistical analysis, "definitely related", "likely to be related" and "possibly related" were analyzed as "related" AE of the research vaccine; "possibly unrelated" and "definitely unrelated" were analyzed as "unrelated" AE.

As the investigetors' understanding of AE / SAE is a gradual process, the initial report may provide inaccurate correlation information. With the increase or update of information in the follow-up visit, investigetors may change their initial judgment on the correlation. At this time, the AE / SAE should be tracked, especially the corresponding information should be updated during the follow-up of SAE

11.8.4.3 ANTICIPATION

The investigator and the sponsor should determine whether AE is expected or unexpected. If the nature, severity or frequency of an AE is inconsistent with the risk information of the previously described study intervention, it should be considered unexpected.

11.9 BIOLOGICAL SAMPLE PROCESSING AND TESTING

11.9.1 BIOLOGICAL SAMPLE PROCESSING

11.9.1.1 Blood sample

Blood biochemical and blood routine laboratory test samples were collected and sent to the testing laboratory, and the samples were tracked to the completion of the test.

The amount of blood collected by humoral immunity test is about 8 ml. hemolysis should be avoided in blood sample collection. After centrifugation, the serum was divided into 8 tubes (EP tubes), each tube was about 250 μ L. the remaining serum was divided into EP tubes, and each tube was 250 μ L for backup.

The amount of blood collected by cellular immunoassay is about 30ml. The venous blood collected by anticoagulant vacuum blood collection vessel is divided into three tubes; after collection, it is required to gently reverse for several times and mix evenly to avoid blood clot. The separated lymphocytes are used for the detection of cellular immunological indicators. Meanwhile, about 3ml of plasma generated during the separation process is collected and divided into 10 tubes, about 0.3ml each tube, 6 tubes are sent for inspection, and 4 tubes are stored as backup. The detection method for replacing pseudovirus/live virus

neutralizing antibody replace method research (competitive ELASA neutralizing antibody detection method) and other immunological methodology research.

The specific procedures and methods of centrifugation, subpackage, cryopreservation and transportation of humoral and cellular immune blood collection are shown in SOP.

11.9.1.2Urine sample

The amount of urine collected in laboratory was about 10.0ml for routine urine test. About 5.0-10.0ml urine samples were collected for urine pregnancy test before (on) the day of vaccination. The urine sample should be treated in time after detection, and no need to be preserved.

11.9.2 BIOLOGICAL SAMPLE NUMBERING RULES

In principle, the serum samples of the subjects were numbered with different sampling time, and the specific number was implemented according to the relevant SOP.

11.9.3 BIOLOGICAL SAMPLE TESTING

The testing quality control standard is provided by the Chinese Academy of inspection. As there is no approved reagent or general method for the detection of RBD antibody and neutralizing antibody of new coronavirus, the detection quality control material shall be solved by the sponsor and the Chinese Academy of inspection through negotiation. The novel coronavirus (SARS-CoV-2) neutralizing antibody and IgG titer were detected by the central hospital.

12. Data management

12.1 DESIGN OF ECRF

ECRF is designed according to the test steps and follow-up visit specified in the protocol. After the first draft is formed, it needs to be jointly reviewed by the project manager, statistician, scheme writer, sponsor, investigator and other project team members. It conforms to the protocol and follows relevant laws and regulations, and the version control process needs to be fully recorded.

12.2 ECRF FILLING GUIDE

The eCRF filling guide is the specific filling instructions for each page of eCRF table and each data point according to the research plan. Ensure that the clinical trial center obtains

the eCRF and its filling guidelines before the subjects are enrolled, and train the relevant staff of the clinical trial center on the protocol, eCRF filling and data submission process, and the process shall be recorded on file.

12.3 ECRF NOTES

Note eCRF is the annotation of blank eCRF, which records the location of each data item of eCRF and its variable name and code in the database. All data items in eCRF need to be labeled. DM review is required.

12.4 DESIGN OF DATABASE

The database should be established according to the data set name, variable name, variable type and variable length in the annotation eCRF, and the structure and setting of the standard database should be followed as far as possible. After the establishment of the database, the database test shall be carried out, and the database test report shall be issued, which shall be signed and confirmed by the person in charge of data management.

12.5AUTHORITY ASSIGNMENT

According to different roles, the system administrator creates accounts and grants different permissions.

12.6 ECRF FILLING

Researchers should collect the data of subjects according to the requirements of the research protocol, and fill in the ECRF accurately, timely, completely and normatively according to the original data and the filling guide. The modification of ECRF data must follow the standard operating procedures and keep the modification trace.

12.7 SENDING AND RESOLUTION OF QUERIES

DM, the data management department, writes a detailed data verification plan. The verification plan is reviewed by the sponsor, medical staff, statistician, project manager, etc. after no objection, the data manager, data manager and the sponsor sign for confirmation. After the data is entered into EDC, the system will edit and check the data according to the data verification plan Check) will check the data, and the data in question will be automatically sent to the system query; the data that cannot be set as the system query will be sent by EDC, and the entry personnel or researchers will confirm and answer the manual

query and system query, and modify the wrong data if necessary until the query is solved. When the answer fails to solve the query, the data manager and monitor can query the data point again, and all the traces are saved in the EDC database.

12.8 DATA MODIFICATION AND REVIEW

Data entry personnel or researchers can modify the data after verifying the data. The modified data should be filled in the system according to the system prompts. Investigators have the right to review all final data.

12.9MEDICAL CODING

AE collected in clinical trials should be coded using a standard dictionary. The standard dictionary commonly used is MedDRA. The encoded data set should clearly record the dictionary and version used when coding.

12.10 SAE CONSISTENCY COMPARISON

All SAE related data points in eCRF are compared with the data points in PV (pharmacovigilance) system by program. Inconsistent data needs to be communicated with PV personnel until there is no difference in data.

12.11 DATA REVIEW MEETING

efore the database was locked, the data manager drafted the draft of the data review report and all the data lists. The sponsor, investigators, data management personnel and statistical analysts jointly reviewed the database, and the statistical analysis population was divided according to the clinical trial protocol check the SAE report and processing records, etc., and finalize the data audit report and population division plan resolution after the data review meeting.

12.12 DATABASE LOCKING AND UNLOCKING

Database locking is an important milestone in the process of clinical research. Locking process and time should be clearly documented. Locking is to cancel the right to edit the database. Any unauthorized account cannot operate the database.

If there is any modification after the database is locked, an application shall be submitted, which can be implemented after discussion and signature of the sponsor, investigator, entry

personnel, clinical supervisor and data management personnel, and the reasons for unlocking shall be recorded in detail.

13. Statistical considerations

13.1 RESEARCH HYPOTHESIS

Not applicable.

13.2 END POINT OF STUDY

The end point of this study is defined in Chapter 7.

13.3 SAMPLE SIZE CALCULATION

According to the requirements of "technical guidelines for clinical trials of vaccines": phase I clinical trial is a small-scale study (20-30 people). In the evaluation of human safety, high and low doses were used to observe the clinical tolerance. A total of 50 subjects were included in this study.

13.4 ANALYSIS SET

Full Analysis Set（FAS）： All subjects who followed the principle of intention to treat (ITT) and were randomized into groups, received at least one dose of vaccine, completed immunogenicity blood collection before immunization and had effective immune evaluation indexes. According to the ITT principle, the immunogenicity was evaluated according to the randomized grouping.

Per Protocol Set 1（PPS1）： All subjects were randomized into groups, completed the first dose of immunization, received immunogenicity blood collection before and 14 days after the first dose of vaccination, and had effective immune evaluation indexes. Among them, subjects who meet the following conditions are not allowed to enter PPS1:

- Those who violate the test protocol before immunogenicity blood collection 14 days after the first dose of vaccination;
- The first dose of vaccine number is wrong;
- 14 days after the first dose of vaccination and before immunogenicity blood collection, those who use the vaccine or drug prohibited by the protocol;
- Other conditions affecting the immunogenicity evaluation 14 days after the first dose

of vaccination.

Per Protocol Set 2（PPS2）： All subjects were randomized into groups, completed the first dose of immunization, completed the immunogenic blood collection before and after the second dose of vaccination, and had effective immune evaluation indicators. Among them, subjects who meet the following conditions are not allowed to enter PPS2:

- Those who violate the test protocol before immunogenicity blood collection before the second dose vaccination;
- The first dose of vaccine number is wrong;
- Those who use vaccines or drugs prohibited by the scheme before the second dose vaccination and immunogenicity blood collection;
- Other conditions affecting the immunogenicity evaluation of the second dose before inoculation.

Per Protocol Set 3，PPS3： All subjects were randomized into groups, completed the first two doses of immunization according to the program, and blood samples were collected 7 days after the completion of PPS3 immunization and the second dose of vaccination, and had effective immune evaluation indexes. Among them, subjects who meet the following conditions are not allowed to enter PPS3:

- Those who violate the test protocol before immunogenicity blood collection 7 days after the second dose vaccination;
- Those with wrong vaccination number of the first two doses of vaccine;
- 7 days after the second dose of vaccination and before immunogenicity blood collection, those who use vaccines or drugs prohibited by the scheme;
- Other conditions affecting the immunogenicity evaluation 7 days after the second dose vaccination.

Per Protocol Set 4，PPS4： All subjects randomized into groups, having completed the first dose of immunization, having completed the immunogenic blood collection before and after the third dose of vaccination, and having effective immune evaluation indicators. Among them, subjects who meet the following conditions are not allowed to enter PPS4:

- Those who violate the test protocol before immunogenicity blood collection before the

third dose vaccination;

- The vaccine number is wrong;
- Those who use vaccines or drugs prohibited by the scheme before the third dose vaccination and immunogenicity blood collection;
- Other conditions affecting the immunogenicity evaluation of the third dose before inoculation.

Per Protocol Set 5，PPS5： All subjects randomized into groups, having completed the first three doses of immunization according to the program, having completed immunogenicity blood collection before vaccination and 7 days after the third dose of vaccination, and having effective immune evaluation indexes. Among them, subjects who meet the following conditions are not allowed to enter PPS5:

- Those who violate the test protocol before immunogenicity blood collection 7 days after the third dose vaccination;
- Those with wrong vaccination number of vaccine;
- 7 days after the third dose of vaccination and before immunogenicity blood collection, those who use vaccines or drugs prohibited by the scheme;
- Other conditions affecting the immunogenicity evaluation 7 days after the third dose vaccination.

Per Protocol Set 6，PPS6： All the subjects were randomized into groups, completed the whole course of immunization according to the program, took immunogenic blood samples before and one month after the immunization, and had effective immune evaluation indexes. Among them, subjects who meet the following conditions are not allowed to enter PPS6:

- Those who violate the test protocol one month after vaccination and before immunogenicity blood collection;
- Those with wrong vaccination number;
- Those who use the vaccine or drug prohibited by the protocol after the whole vaccination and before the immunogenicity blood collection one month;

- Other conditions affecting the immunogenicity evaluation one month after the whole vaccination.

FAS, PPS1, PPS2, PPS3, PPS4, PPS5 and PPS6 are mainly used to evaluate the immunogenicity of vaccines.

Immune Persistence Set 6，IPS6： Including all subjects who completed the whole course of immunization, blood collection for immune persistence evaluation 6 months after the completion of the whole vaccination, and had effective immune evaluation indicators.

IPS6 is mainly used for the evaluation of immune persistence 6 months after the whole course of vaccination.

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

In this study, the safety analysis of each dose was based on the actual number of vaccinated people, that is: the safety set of the first dose includes all the subjects vaccinated with the first dose of experimental vaccine, which is recorded as SS1; the safety set of the second dose includes all the subjects vaccinated with the second dose of the trial vaccine, which is recorded as SS2; the safety set of the third dose includes all the subjects vaccinated with the third dose of the trial vaccine, which is recorded as SS3.

The above analysis set will be discussed and decided by the principal investigator, the sponsor, the statistician and the data manager at the data blind review meeting before the database is locked.

13.5 STATISTICAL ANALYSIS METHOD

13.5.1GENERAL PRINCIPLES

The measurement data were statistically described by means, median, standard deviation, maximum value and minimum value; count data or grade data were expressed by frequency and frequency.

All statistical analysis was performed by SAS 9.4.

13.5.2 DEMOGRAPHIC AND BASELINE CHARACTERISTICS

The number of subjects who were screened, enrolled and completed the experiment, as

well as the number of subjects in each analysis set were summarized, and the reasons for the dropping off of the subjects were analyzed. Demographic and baseline characteristics of the subjects were statistically described. A list of screening failed subjects, dropped subjects and those who did not enter each analysis set were listed.

13.5.3 IMMUNOGENICITY ANALYSIS

The positive rates of novel coronavirus (SARS-CoV-2) neutralizing antibody, S protein binding antibody (IgG) and RBD protein binding antibody (IgG) will be calculated at 14 days after the first dose of vaccination, before the second dose of vaccination, 7 days after the second dose of vaccination, before the third dose of vaccination, 7 days after the third dose of vaccination (if applicable), 1 month after the whole course of vaccination and 6 months after the whole course of vaccination, and the two-sided 95% confidence intervals are calculated by Clopper-Pearson method. Chi-square test / Fisher exact probability method is used to test the difference between groups.

The levels of neutralizing antibody to sars-cov-2, S protein binding antibody (IgG), RBD protein binding antibody (IgG), and the fold increase relative to pre-vaccination in each group are statistically described by geometric mean and 95% confidence interval (95% CI) at 14 days after the first dose of vaccination, before the second dose of vaccination, 7 days after the second dose of vaccination, before the third dose of vaccination, 7 days after the third dose of vaccination (if applicable), 1 month after the whole course of vaccination and 6 months after the whole course of vaccination. Log-transformed analysis of variance is used to test the difference between groups.

The levels of IL-2, IL-4, IL-5, IL-6 and IFN - γ and their changes from pre-vaccination are statistically described at 14 days after the first dose of vaccination, before the second dose of vaccination, 7 days after the second dose of vaccination, before the third dose of vaccination (if applicable), 7 days after the third dose of vaccination, 1 month after the whole course of vaccination and 6 months after the whole course of vaccination. The K-W test is used to tes the differences between groups at the above time points.

13.5.4 SAFETY ANALYSIS

13.5.4.1 AE AND SAE

AE and SAE were coded by MedDRA, and classified and counted according to SOC and

Pt. In addition, according to the provisions of the program, the collection of adverse events will be classified and counted according to the systemic and local reactions. In this study, Treatemnt Emergent Adverse Event (TEAE) occurred after the first dose of vaccination were statistically analyzed, that is, the AE occurred after the first dose of vaccination or aggravated after the first dose of vaccination. The AE occurred before the first dose of inoculation was listed in the form of a list. AE below is a TEAE unless otherwise specified.

The occurrence, number and incidence of all AE, AE related to research vaccine, AE unrelated to research vaccine, grade 3 and above, AE related to research vaccine, AE leading to withdrawal, and AE related to research vaccine leading to withdrawal were calculated respectively. The dose distribution, time distribution and severity of adverse events were statistically described. List the AE related to the research vaccine, the ae not related to the research vaccine, the AE list with level 3 and above, and the AE list leading to quit.

The incidence, number and incidence of all SAE, SAE related to the vaccine and SAE not related to the vaccine were calculated respectively. Make a list of SAE.

13.5.4.2 LABORATORY EXAMINATION

Statistical description was made on 7 days after each dose of inoculation and its changes compared with those before inoculation; the difference among groups was statistically tested by analysis of variance. The changes of clinical significance of laboratory tests (including blood routine test, blood biochemistry test and urine routine test) before and after inoculation were listed in the form of cross table (according to the normal range and the researcher's judgment of clinical significance). List the abnormal results of laboratory examination after the first dose of inoculation.

13.5.5 SUBGROUP ANALYSIS

Subgroup analysis was not planned for this study.

13.5.6 MULTIPLICITY ISSUES

This trial is a phase I dose exploration test and does not involve multiple issues. In the results of immunogenicity evaluation and safety evaluation, the calculated p value is only nominal p value, which is mainly used to describe the strength of association between evaluation endpoint and treatment group, but not as the basis for formal statistical inference.

13.5.7 PROCESSING OF MISSING DATA

In the evaluation of the immunogenicity of FAS, the missing serum test results were filled by the method of last observation carried forward (LOCF). The missing data in the safety endpoint were no longer processed in this study.

13.5.8 STATISTICAL ANALYSIS STRATEGY

The safety data and immunogenicity data were analyzed one month after the whole vaccination, and the statistical analysis report and summary report were prepared to facilitate the communication between the sponsor and CDE

One year after the whole vaccination, the remaining safety data and immune persistence data were analyzed, and the statistical analysis report and summary report were prepared.

14. Subject safety and adverse event management

14.1SAFETY PRECAUTIONS

Clinical trials were conducted in medical units with vaccination qualification at county and municipal level. Before the start of the trial, the sponsor assessed the research site in strict accordance with the requirements of GCP, focusing on whether the environmental facilities of the test site meet the requirements of *the guiding principles for quality management of clinical trial of vaccines (Trial)*, the first-aid facilities and first-aid equipment in the first-aid room are effective, and the first-aid medicine is within the validity period, and the first-aid doctor has the corresponding qualification and ability. When AE occurred on the test site, the subjects were treated in the on-site emergency room in a timely manner. After the condition was stable, the subjects were sent to the emergency department of the hospital for treatment. During the period of admission, the subjects should inform the hospital for timely treatment. Strict SOP should be adopted to stipulate the responsibilities, contact telephone number and rescue route of the staff to ensure the timely treatment of sudden AE, and to ensure the effective contact between the subjects and the investigators, so that any AE can be reported and dealt with quickly. When the subject needs emergency treatment after SAE, the hospital can provide green channel services such as medical treatment, hospitalization and medical security to ensure the timely treatment of the subjec

The sponsor shall appoint full-time personnel to be responsible for the safety information monitoring of clinical trials and the management of SAE reports. The sponsor and the

investigator shall formulate the standard operating procedures for safety information monitoring and SAE report of clinical trials, and train all relevant personnel. The AE monitoring and reporting of vaccine clinical trials were completed by subjects, adverse event investigators and researchers at different observation time points in different stages.

14.2DISCOVERY AND COLLECTION OF ADVERSE EVENTS

The subjects were asked whether they received hospitalization, outpatient treatment or self medication for any reason, and the information was recorded.

During the training of subjects, it is emphasized that AE should be reported in time. Researchers should be highly alert to such incidents and investigate and deal with them in time.

When an SAE occurs, it is the responsibility of the investigator to review all documents related to the event (e.g. hospital course and order records, laboratory reports and diagnostic reports), or arrange clinical examinations / tests as required by the sponsor in order to clarify the nature and relevance of SAE. If a subject is confirmed dead during the study period or during the follow-up period, the hospital's final conclusions on the deceased should be collected, and copies of the results, including histopathological results, should be obtained if autopsy is performed.

Investigators should collect complete case copies as far as possible, but can not replace the research records with the copies of subjects' cases. All information related to SAE should be recorded on the original records, ECRF and SAE report pages.

If medical records should be made public for medical identification, all the content columns that can identify the subjects should be covered before disclosure.

14.3SERIOUS ADVERSE EVENT REPORT

14.3.1REPORTING TIME

Researchers should fill in the first report of "SAE report form" within 24 hours after being informed of SAE, and report to the main investigator, sponsor, ethics committee, provincial drug regulatory agency, and monitor of CRO company in the form of fax, email or Internet. After completing the first report, the sponsor and researcher should continue to follow up SAE, timely submit relevant new information or changes to the previous report, event outcome, etc. in the form of follow-up report, pay attention to the SAE that is not cured

/ not recovered or is recovering / recovering until recovery, and complete the summary report.

In order to ensure the timely reporting of SAE, the receiving unit, personnel, address, fax number and email address of SAE should be clearly listed in the clinical trial SOP.

14.3.2REPORT CONTENT

1) Report type and reporting time (first report, follow-up report, summary report and corresponding reporting time);

2) Subject information (abbreviation, random number, date of birth, gender);

3) Information of the reporter (medical institution and professional name, telephone number, reporter's position / Title);

4) Information of suspected drugs (Chinese and English drug names, registration classification and dosage forms);

5) Study related information (clinical research approval number, clinical research classification, clinical trial indications);

6) Information of combined diseases and treatment (diagnosis name, treatment drug name, usage and dosage);

7) Detailed information of SAE (diagnosis name, severity criteria, occurrence time, end time, laboratory test results, treatment process, outcome, measures taken for the research vaccine and its correlation with the vaccine, etc.);

8) The time when the investigator was informed;

9)Signature of the investigator.

14.4SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION， SUSAR

Suspected Unexpected Serious Adverse Reaction (SUSAR)should meet the following three criteria: (excerpted from "*Regulations on the management of severe adverse events in vaccine clinical trials (Trial)*")

1) Suspicious adverse reaction: it refers to the adverse reaction that has nothing to do with the purpose of medication at any dose, which is considered to be at least possibly related to the drug after analysis.

2) Unexpected adverse reaction: refers to the nature, degree, consequence or frequency

of adverse reaction, which is different from the expected risk described in previous protocol or other relevant information (such as the researcher's manual and other documents). As the main document, the investigator's manual provides safety reference information to judge whether an adverse reaction is expected or not.

3) Serious adverse reaction: it refers to one of the following situations: causing death; endangering life; causing cancer, teratogenesis, birth defects; causing disability or organ function damage, affecting working ability; causing hospitalization or prolonging hospitalization time; leading to other important medical events, such as without treatment, the above situations may occur It's not.

After being informed of SUSAR, the sponsor shall immediately conduct comprehensive analysis, evaluation and judgment. According to the nature (category) of the event, the first report shall be submitted to the drug evaluation center of the State Drug Administration and the local provincial drug supervision and administration department within the following time limit, and the principal investigator and main collaborative researchers of all relevant clinical trial institutions shall be informed:

1) For fatal or life-threatening suspicious and unintended serious adverse reactions (SUSAR), the sponsor should report it as soon as possible but not more than 7 natural days after the first notification, and report the relevant follow-up information within 8 natural days thereafter.

2) For non lethal or life-threatening suspicious and unintended serious adverse reactions (SUSAR), the sponsor should report as soon as possible after the first notification, but not more than 15 natural days.

3) For other potential serious safety risk information, the sponsor should report as soon as possible after the first information, but not more than 15 natural days.

At the same time, the rapid report should also be carried out according to the specific requirements of "*standards and procedures for rapid reporting of safety data during drug clinical trials*". When the sponsor and the researcher can not reach an agreement in the judgment of causal relationship between AE and vaccine, the judgment of either party can not exclude those related to the vaccine, and the rapid report should also be conducted.

14.5TREATMENT AND MANAGEMENT OF ADVERSE EVENTS

The investigators should establish the emergency plan for SAE treatment in clinical trials, train all relevant personnel, take measures to timely inform the subjects of any clinically significant diseases / events after vaccination, and make the subjects timely go to the designated hospital for appropriate treatment in accordance with the relevant national regulations and current medical management norms. Drugs used to treat AE should be recorded in the subject's original record and eCRF.

In case of disagreement and dispute in AE treatment, the investigator is obliged to cooperate with the sponsor and assist the subject in medical identification.

The sponsor has the obligation and responsibility to unconditionally ensure the safety of the subjects, and provide humane care and compensation to the subjects who have AE related to the research vaccine during the clinical trial.

The investigators should pay continuous attention to the AE that continues because of the termination of AE or the end of the visit. The AE related to vaccination should be followed up until the end of the event. The follow-up can be stopped if the unrelated events such as diseases are diagnosed by doctors.

15. Quality assurance and monitoring of clinical trials

15.1 INVESTIGATORS

The organization in charge of vaccine clinical trial should establish perfect organization management system and quality management system, have management mechanism and measures to prevent and deal with emergencies in vaccine clinical trial, have SAE emergency response expert team and technical ability to deal with SAE, and have perfect cold chain equipment for vaccine delivery and storage. The trial site of vaccine clinical trial has the vaccination qualification approved by the health and family planning administrative department, has a relatively fixed and sufficient number of clinical trial researchers, is equipped with the standard operating procedures related to vaccine clinical trial, conducts training and has training records, and establishes the SAE medical treatment green channel for vaccine clinical trial. According to the vaccination and visit process of vaccine clinical trial, the functional divisions are set up, including reception area, informed consent room, inquiry and physical examination screening room, biological specimen collection room, vaccination

room, emergency room, medical observation room, vaccine storage room, archives room, sample processing and preservation room, case screening laboratory and temporary storage place of medical waste, etc., to establish a green channel for first aid Ambulances, relevant rescue personnel and first-aid supplies are available at the inspection site. All the researchers should have clear division of work and be authorized to carry out the project, and all the researchers should be qualified and qualified. The researchers in charge of the institution and the experimental site were trained in GCP and vaccine clinical trial technology, and had training records. Supporting personnel shall have records of participating in corresponding work training.

15.2 SPONSOR

The sponsor is ultimately responsible for the quality of clinical trials. We should establish a perfect vaccine clinical trial quality management system, formulate corresponding SOP, organize the inspection of clinical trial, and conduct systematic inspection of clinical trial related activities and documents, including the test site, laboratory, CRO company, etc., to evaluate whether the test is carried out according to the test scheme, SOP and relevant laws and regulations, and whether the test data is timely, true, accurate and complete record. The audit was performed by personnel not directly involved in clinical trials.

The trial site should cooperate with the inspection of clinical trial items, keep relevant records, formulate improvement plans for problems found in the audit, and take corresponding management measures to improve the quality of the trial.

15.3 MONITOR

According to Article 50 of "*the guiding principles for quality management of vaccine clinical trials (Trial)"*, the sponsor shall appoint a sufficient number of monitors to supervise the whole process of clinical trials. The supervisor should have educational background and working experience in medicine, pharmacy or related specialties. The number of monitors designated by the sponsor for vaccine clinical trial should be determined according to the frequency of the test and the complexity of the design of the trial protocol. The monitor should supervise the clinical trial according to the requirements of the audit plan and submit the audit report.

The monitor supervises the whole process of clinical trial to ensure that the implementation of clinical trial meets the requirements of trial protocol, SOP, GCP and relevant laws and regulations, and is completed as expected.

15.4 BIOLOGICAL SAMPLE MANAGEMENT

The samples for laboratory testing are managed by special personnel and sent to the testing laboratory at room temperature, and the samples are tracked until the test is completed.

The blood samples used for immunogenicity test shall be managed by special personnel, and sample preservation and temperature record shall be established. The storage temperature shall be monitored and recorded every working day (under the premise of automatic temperature monitoring and alarm, it can be arranged according to the specific situation on site during holidays).

The serum samples should be sent to the testing laboratory under the condition of cold storage (dry ice / low temperature ice arrangement / other freezing methods). The backup blood samples should not be transported at the same time. Theinvestigetors should be responsible for keeping them properly until the immunogenicity test (if necessary) or after the clinical report is completed, and then processed after confirmation by the sponsor.

The validity period of whole blood for cellular immune test is less than 24 hours. Blood samples after laboratory test will be processed according to the contract or requirements of the sponsor. The blood samples that need to be processed will be executed according to the SOP of "*biological sample disposal and management sequencing*".

During the whole process, the handover management should be carried out strictly, and the researchers, sample transporters and laboratories should keep records properly.

15.5 VACCINE MANAGEMENT

The organization in charge of vaccine clinical trial shall guide the trial site to formulate the management system of research vaccine, and the management of receiving, keeping, preparing, recycling, returning / destroying of research vaccine shall meet the requirements of relevant laws and regulations. Both the organization in charge of vaccine clinical trial and the trial site shall appoint personnel who have received GCP and relevant training to be responsible for the management of research vaccine.

Vaccine delivery: the whole process of vaccine management should meet the requirements of cold chain, and there should be conditions for vaccine transportation and storage that meet the requirements of the scheme. The vaccine shall be stored and transported away from light at 2-8 ℃. The delivery process of vaccine shall be provided with delivery note and temperature monitoring. The packaging and unpacking temperature shall be recorded upon arrival. The receiver shall sign and fax or copy the delivery note to the consignor after receiving the vaccine, and both parties shall keep the delivery note properly.

Storage, distribution and use of vaccine: the research vaccine shall be managed according to the requirements of independent partition and special cabinet locking. The vaccine recipient must verify the delivery status of the vaccine, establish a vaccine handover, registration, use and recycling work form, fill in as required, and keep it in the work record.

Vaccine handover record: the sponsor shall provide the vaccine, and the researcher shall verify the name, quantity and package of the vaccine at the same time, and make the handover record.

Vaccine registration and use records: the researchers shall establish vaccine registration and use records. The use records of vaccines distributed to each subject should be kept, including the study number, the subject's initials, and the vaccinator's signature.

Vaccine recovery record: the vaccine administrator shall timely recover the remaining vaccine, conduct regular inventory and complete the inventory record. If the vaccine use and surplus quantity are inconsistent with the total number, the situation shall be explained. The abandoned, expired and remaining vaccines were returned to the sponsor. When the sponsor receives the vaccine, it shall check the quantity of vaccine and make relevant records, which shall be signed by the vaccine administrator and the representative of the sponsor.

Cold chain damage: once the temperature abnormality of < 2 ℃ or > 8 ℃ occurs in the refrigerator storing vaccine, it is regarded as cold chain damage. The sponsor should explain the information related to the stability of the vaccine in the researcher's manual. In case of cold chain damage, the researcher should transport the vaccine to 2-8 ℃ dark environment for storage, stop using the cold chain damaged vaccine, report to the sponsor as soon as possible, and decide to stop or continue to use the vaccine according to the written opinions of the sponsor. The cold chain damaged vaccine should not be used in the subjects until the sponsor's advice has been obtained.

The vaccine under study is not allowed to be used in non clinical trial population.

15.6 CALIBRATION OF INSTRUMENTS AND EQUIPMENT

- The thermometer used to monitor the temperature of the refrigerator has been calibrated. Within the validity period, the refrigerator must have three consecutive days of normal temperature monitoring records before use;
- The thermometer has been standardized;
- The syringes for vaccine injection and blood collection are disposable sterile syringes. The manufacturer has the national production license and records the batch number and expiration date;
- The cold storage equipment used to store vaccines and samples on site is in use after annual inspection;
- Height meter, weight scale, blood pressure meter and other measuring instruments are qualified and in use.

15.7 ORIGINAL DATA MANAGEMENT

The original data such as informed consent, original medical record, contact card and SAE report form are the important basis for the traceability of clinical trials, which should be recorded timely, accurately, completely, standardized and truly, and properly kept in the research site.

Authorized and specially trained researchers shall input the research data into eCRF according to the original data, which shall not be changed at will. If there is any error in the input, it shall be modified according to the filling guide. In order to ensure the authenticity and reliability of clinical trial data, the reviewer and the investigator jointly review eCRF. After the investigator signs, all data are processed by the statisticians entrusted by the clinical trial responsible unit or the sponsor unit.

15.8 RESEARCH MATERIALS

The sponsor and the research party provided clinical trial data in accordance with the "*drug registration management measures* "and GCP.

The researcher's folder shall be arranged according to the requirements of GCP and kept in the research party, who shall be responsible for sorting out and summarizing the data

delivered to the sponsor. Materials recording real information of subjects, such as screening registration form, informed consent form, original medical records, contact cards, medical records of subjects, etc., were sealed in the test site. The coordinator / Archivist of the responsible institution and the on-site archivist should check and hand over the information, and both parties should sign the storage agreement or memorandum.

The file management shall be carried out according to SOP, and the identification labels including project name, completion date, application unit and storage period shall be made, and safety measures such as insect prevention, moisture prevention, fire prevention and anti-theft shall be taken. The use and access of the project data is limited to the relevant personnel of the project, relevant personnel of the sponsor (including project monitors) and NMPA project inspectors. All materials shall be kept for five years from the date of site closure, and the sponsor shall be informed after the expiration of the period. No one is allowed to handle the information without authorization before receiving the written notice from the sponsor.

15.9 AGREEMENT AND CLINICAL SUMMARY REPORT

The responsibilities of all parties in the clinical trial shall be stipulated in the agreement, which shall come into force after being signed and sealed by all parties concerned. The original of the agreement is made in 4 copies, 2 copies for the sponsor and 2 copies for the research party.

Clinical summary report (red seal): in 5 copies, 1 copy for the research party and 4 copies for the sponsor to apply for the new drug certificate and approval number. Copies can be kept on the research site.

16.Ethics committee

16.1 REVIEW CLINICAL TRIALS

The ethics committee needs to review the scientificity and ethical rationality of drug clinical trials, so as to ensure the dignity, safety and rights of subjects, promote the scientific and healthy development of drug clinical trials, and enhance public trust and support for drug clinical trials.

The ethics committee of the institution in charge of clinical trial can review the trial protocol, informed consent, recruitment materials and other written materials provided to the subjects respectively. The revision of the Protocol shall be negotiated with the sponsor. If the

content of the informed consent is not inconsistent with the protocol and conforms to the local actual situation, the opinions of the ethics committee can be adopted.

16.2 IMPLEMENTATION PROCESS REVIEW

16.2.1 FOLLOW UP REVIEW

All approved clinical trials shall be followed up by the ethics committee until the end of the trial.

16.2.2 AMENDMENT REVIEW

Any modification to the trial protocol during the clinical trial shall be submitted to the Ethics Committee for review and approval / filing before implementation. The ethics committee shall request the sponsor and / or researcher to submit relevant information for the review of the amendment, including (but not limited to) the following:

（一）The content and reason of modification;

（二）The impact of the revised scheme on the expected risks and benefits;

（三）The effect of the modified protocol on the rights and security of the subjects.

The ethics committee mainly evaluates the risks and benefits of the modified scheme and makes review opinions. In order to avoid causing emergency injury to the subjects, the researcher can implement the protocol before submitting it to the Ethics Committee for examination and approval, and make a written report to the ethics committee in time afterwards.

16.2.3 ANNUAL / PERIODIC FOLLOW-UP REVIEW

The frequency of annual / periodic follow-up review shall be determined by the ethics committee at least once a year according to the risk degree of the test. The ethics committee shall require researchers to submit reports on time. The annual / regular follow-up review report information includes (but is not limited to) the following:

（一）The progress of the trial;

（二）The number of subjects included, completed and quit;

（三）Confirm that serious adverse events are reported in time and handled properly;

（四）Any event or new information that may affect the benefit of the research risk.

After reviewing the progress of the study, the ethics committee reassessed the risks and benefits of the trial.

16.2.4 REVIEW OF SERIOUS ADVERSE EVENTS

The ethics committee shall review the SAE reported by the sponsor and / or researcher, including the extent and scope of SAE, the impact on the risk benefit of the trial, and the medical protection measures of the subjects.

16.2.5 REVIEW OF NON-COMPLIANCE /VIOLATIONS

In case of non-compliance / violation of the protocol during the clinical trial, the ethics committee shall require the sponsor and / or investigator to explain the cause, impact and treatment measures of the event, and review whether the event affects the safety and interests of the subjects and the risk benefit of the trial.

16.2.6 EARLY TERMINATION OF TRIAL REVIEW

If the sponsor and / or investigator needs to apply to the Ethics Committee for review in case of early termination of the trial, the ethics committee shall require the sponsor and / or researcher to report the reasons for early termination of the trial and the follow-up treatment of the subjects, and examine whether the safety and rights and interests of the subjects are guaranteed.

16.2.7 FINAL INSPECT

The ethics committee should ask the sponsor and / or investigator to report the completion of the trial and review the protection of the safety and interests of the subjects.

17. Publication of papers

After the completion of the trial, the research unit may publish the summary report or research results related to the clinical trial in the form of a paper after obtaining the written authorization of the sponsor. The researcher of the research unit and the technical cooperation unit (pharmacodynamics evaluation) shall have the right of authorship. Negative or inconclusive research results should also be published or published as positive results.

18. References

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17) NIH-FDA IND/IDE Phase II and Phase III clinical trial protocol template (Chinese translation), the First Vaccine Clinical Trial Center of Peking University, June 12, 2017

18) *Measures for the Identification of Abnormal Responses to Vaccination*, the National Health Commission of the PRC, December 1, 2008

19) *Guidelines for Vaccination Work*, the National Health Commission of the PRC, December 6, 2016

20) Vaccine storage and transportation management regulations (2017 edition), the National Health Commission of the PRC

21) Yanping Zhang. Analysis of the epidemiological characteristics of novel coronavirus pneumonia. Chinese Journal of Epidemiology. 2020. 41(2): 145.

Appendix 1: list of blood collection and inspection items involved in the protocol

Laboratory inspection items	Inspection details	Visit viewpoints
routine blood test	White blood cell, platelet, hemoglobin, lymphocyte	V1, V2, V3, V6, V7, V9, V10
Blood biochemistry	Alanine aminotransferase, aspartate aminotransferase, total bilirubin, urea, creatinine, fasting blood glucose, creatine kinase (only for V9 and V10), lactate dehydrogenase (only for V9 and V10)	V1, V2, V3, V6, V7, V9, V10
routine urine test	Urine protein, urine red blood cell	V1, V2, V3, V6, V7, V9, V10
Four coagulation items	Prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen	V1
Blood pregnancy (female)		V1
Urine pregnancy (female)		V2, V6, V9
Virus serological examination	Human immunodeficiency virus (HIV) antibody, hepatitis B surface antigen, hepatitis C antibody, syphilis specific antibody	V1
12 lead ECG		V1
Chest CT		V1
Screening of IgM and IgG antibodies against SARS-CoV-2		V1
Real time RT-PCR nucleic acid screening for Sars-		V1, V2, V6, V9, V14

Cov-2		

Randomized, Double-Blind, Placebo-Controlled Phase Ⅱ Clinical Trial to Evaluate the Immunogenicity and Safety of the Recombinant Novel Coronavirus Vaccine (CHO cells) with Different Doses and Different Immune Procedures in Healthy volunteers Aged 18-59 Years

Product name：	Recombinant novel coronavirus vaccine (CHO cells)
Drug clinical trial approval:	2020L00023、2020L00024
Approval time：	June 19th, 2020
Registration category：	Preventive biological products
Protocol Number：	LKM-2020-NCV02
Version date：	July 13, 2020
Version number：	Version 2.1

Sponsor (stamped)	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.
Research unit (seal)	Hunan Provincial Center for Disease Control and Prevention
Research time：	July 2020 to November 2021

Sponsor approval

Protocol Number	LKM-2020-NCV02
Version date	July 13, 2020
Version number	Version 2.1
Study Title	Randomized, Double-Blind, Placebo-Controlled Phase Ⅱ Clinical Trial to Evaluate the Immunogenicity and Safety of the Recombinant Novel Coronavirus Vaccine (CHO cells) with Different Doses and Different Immune Procedures in Healthy volunteers Aged 18-59 Years
Sponsor Project Manager	Name: Shilong Yang Company: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. Address: No.100 Fushan Road, High-tech Zone, Hefei City, Anhui Province Postcode: 230088 Tel：15155935085 Email：yangshilong@zhifeishengwu.com
Sponsor Contact	Name: Zaixin Zhong Company: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. Address: No.100 Fushan Road, High-tech Zone, Hefei City, Anhui Province Postcode: 230088 Tel: 18130032795 Email：zhongzaixin@zhifeishengwu.com
Protocol Approval	Signature: Date of approval: (mm / dd / yyyy)

Principal Investigator Approval

Protocol Number	LKM-2020-NCV02
Version date	July 13, 2020
Version number	Version 2.1
Research topic	Randomized, Double-Blind, Placebo-Controlled Phase Ⅱ Clinical Trial to Evaluate the Immunogenicity and Safety of the Recombinant Novel Coronavirus Vaccine (CHO cells) with Different Doses and Different Immune Procedures in Healthy volunteers Aged 18-59 Years

I agree:

- Undertake the responsibility of correctly guiding clinical research in this area.
- Ensure that this research is conducted in accordance with the trial protocol and Standard Operating Procedures (SOP) for clinical research.
- Ensure that the personnel participating in this research fully understand the research product information and other research-related responsibilities and obligations specified in this clinical protocol.
- Ensure that no changes are made to the trial protocol without the review and written approval of the sponsor and the Institutional Review Board (IRB) unless it is necessary to eliminate the immediate harm to the subjects or for reasons to comply with the requirements of the drug regulatory agency (for example administrative aspects of the project).
- Familiar with the correct use of the vaccine described in the trial protocol, fully understand the other information provided by the sponsor, including but not limited to the following: the current investigator's manual or equivalent documents.
- Familiar with and abide by the *Vaccine administration law of the People's Republic of China*, *Good Clinical Practice* (GCP), *Guidelines for quality management of vaccine clinical trials (Trial),* and all current regulatory requirements.

Name of Principal Investigator		Unit	

Signature		Date	

Table of Contents

Abbreviation list

English abbreviations	English full name
ADE	Antibody dependence enhancement
AE	Adverse Event
ALT	Alanine aminotransferase
ANC	Neutrophil
Arthus	Local type Ⅲ hypersensitivity reaction
AST	Aspartate aminotransaminase
BAT-SL-COVZC45	Bat SARS-like coronavirus
CI	Confidence Interval
COPD	Chronic obstructive pulmonary diseases
COVID-19	Corona Virus Disease 2019
CPK	Creatine Kinase
CR	Creatinine
CRO	Contract Research Organization
DM	Data management
DSMB	Data Safety and Monitoring Boards
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture System
Eos	Eosinophilic granulocyte
FAS	Full Analysis Set
FEV1%	Forced expiratory volume in one second
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GLU	Blood Glucose
HBGA	Human histo-blood group antigen
HGB	Hemoglobin
HUH-7	Human hepatoma
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IRB	Institutional Review Board

LOCF	Last Observation Carried Forward
LY	lymphocyte
MedDRA	Medical Dictionary for Regulatory Activities
MERSR-COV	Middle East Respiratory Syndrome Coronavirus
NMPA	National Medical Products Administration
PLT	Blood Platelet
PPS	Per Protocol Set
PRO	Urine Protein
PV	Pharmacovigilance
RBC	Red Blood Cell
RBD	Receptor binding domain
SAE	Serious Adverse Event
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome-Corona Virus Disease
SAS	Statistical analysis system
SOP	Standard Operation Procedure
SS	Safety Set
Stevens-Johnsons syndrome	Severe erythema multiforme
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBIL	Total Bilirubin
TEAE	Treatment Emergent Adverse Event
VED	Vaccine enhanced disease
VERO E6	Monkey kidney cell
WBC	White Blood Cell
WHO	World Health Organization

Glossary of terminology

Standard operating procedures	Standards and detailed written procedures for the effective implementation and completion of each task in a clinical trial.
Case report form	A paper or electronic file that is designed in accordance with the requirements of the trial protocol and reported to the sponsor to record relevant information about the subject.

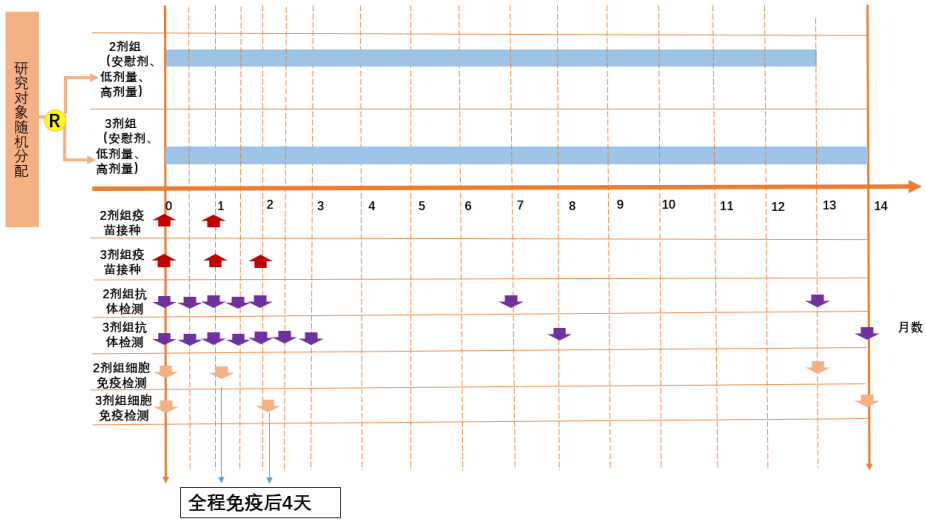
Audit	Conduct systematic and independent inspections of clinical trial-related activities and documents to assess and determine whether the implementation of clinical trial-related activities, the recording, analysis, and reporting of trial data meet the requirements of the trial protocol, standard operating procedures, and relevant laws and regulations.
Monitoring	Supervise the progress of clinical trials, and ensure that clinical trials are implemented, recorded, and reported in accordance with the trial protocol, standard operating procedures, and relevant laws and regulations.
Experimental vaccine	Vaccines used in clinical trials, including trial vaccines and placebos.
Randomization	The principle of randomization in clinical trials refers to the implementation process or measures in which each subject in a clinical trial has the same chance to be assigned to the trial group or the placebo group. The randomization process is not affected by the subjective wishes of the investigator and / or the subject.
Blinding method	Blinding is one of the important measures to control the bias caused by "knowledge of randomization grouping information" in clinical trials, and the purpose is to achieve the unpredictability of randomization treatment grouping by all parties in clinical trials.
Subject	Participate in a clinical trial and be the recipient of the experimental vaccine, including patients and healthy subjects.
Dropout	It means that the subject cannot continue to follow the trial protocol to the required last follow-up for any reason.
Investigator's brochure	The clinical and non-clinical research data of the relevant experimental vaccines in human trials.
Preventive drugs	Drugs used to prevent solicited AEs that may occur during the recruitment period after vaccination.
Informed Consent Form	It is a documentary proof that each subject has indicated that they voluntarily participate in a certain trial. The researcher needs to explain to the subjects the nature of the trial, the purpose of the trial, the possible benefits and risks, other available treatment methods, and the rights and obligations of the subjects in accordance with *the Declaration of Helsinki* so that the subjects express their consent after they fully understand.

Adverse event	All adverse medical events that occurred after subjects received the experimental vaccine, but not necessarily causally related to treatment.
Collective adverse events	Adverse events collected as safety endpoints in clinical studies refer to information on adverse events actively collected by investigators or subjects during a specific follow-up period after vaccination.
Non-collective adverse events	Other adverse events other than collective adverse events reported in clinical studies also include collective adverse events reported outside the designated collective time window.
Serious adverse event	The subject has died, life-threatening, permanent or severe disability or loss of function after receiving the experimental vaccine, the subject needs to be hospitalized or extended hospital stay, and congenital abnormalities or birth defects and other adverse medical events.
Data Safety and Monitoring Board	An independent committee composed of a group of professionals with relevant professional knowledge and experience established by the sponsor which can regularly evaluate clinical trial progress, safety data, and key efficacy indicators, and recommend whether to continue, modify or stop the trial.

Protocol abstract

Research topic	Randomized, Double-Blind, Placebo-Controlled Phase Ⅱ Clinical Trial to Evaluate the Immunogenicity and Safety of the Recombinant Novel Coronavirus Vaccine (CHO cells) with Different Doses and Different Immune Procedures in Healthy volunteers Aged 18-59 Years
Brief Title	Recombinant Novel Coronavirus Vaccine (CHO cells) Phase II clinical trial
Products specifications	Recombinant novel coronavirus vaccine (CHO cells), 25 μg / 0.5 mL / bottle Recombinant novel coronavirus vaccine (CHO cells), 50 μg / 0.5 mL / bottle
Indication	Prevent respiratory diseases caused by novel coronavirus infection
Study population	Healthy volunteer aged 18 to 59
Research unit	Hunan Provincial Center for Disease Control and Prevention
Research Purpose	**Main purpose:** Evaluate the immunogenicity and safety of the novel coronavirus vaccine (CHO cells) with different doses and different immune procedures in healthy volunteer aged 18-59 years **Secondary purpose:** Further, explore the immune durability of the novel coronavirus vaccine (CHO cells) with different doses and different immune procedures
Study Design	**Overall design:** A single-center, randomized, double-blind, placebo-controlled clinical trial. **Immune procedure:** The study is divided into the 0, 1 month vaccination procedure (2-dose group) and the 0,1,2 month vaccination procedure (3-dose group). Before the third dose of vaccination and after the second dose of vaccination, the safety data must be evaluated and approved

	by CDE before the third dose of vaccination can be vaccinated. **Study population:** 900 healthy volunteer aged 18-59 (including 18 and 59 years old) **Trial group：** The 0, 1 month vaccination procedure：the 2-dose group (150 cases in the low-dose group, 150 cases in high-dose group), and 2-dose placebo-controlled group (150 cases); The 0, 1, 2 month vaccination procedure: the 3-dose group (150 cases in the low-dose group, 150 cases in high-dose group), and 3-dose placebo-controlled group (150 cases). **Study plan and implementation：** Volunteers aged 18-59 were asked for their medical history, travel history, residence history, and other information after they signed the informed consent form. Then relevant examinations were performed, including height, weight, vital signs, physical examination (skin, heart, and lung auscultation), urine pregnancy test (Fertile woman, etc. Qualified 18~59-year-old subjects were randomly distributed into the 2-dose group or 3-dose group. Throat swabs were collected for SARS-CoV-2 real-time fluorescent RT-PCR nucleic acid detection, and blood was collected for SARS-CoV-2 IgM and IgG antibody detection. Subjects will be vaccinated according to the 0, 1 month vaccination procedure or the 0, 1, 2 month vaccination procedure. If a suspension/termination criteria event occurs, the DSMB will discuss whether to terminate or continue the trial.



Safety assessment:

AE and SAE: Collect all adverse events (AE) 30 minutes after each dose, all AEs (including collective and non-solicited AEs) from 0-7 days, and all AEs (non-solicited AEs) from 8-30 days after vaccination, all serious adverse events (SAEs) in the next 12 months from the first dose to the entire vaccination.

Solicited AE (the following events occurred within day 7 post-vaccination) :

Inoculation site (local) adverse reactions: pain, swelling, induration, redness, rash, pruritus

Vital signs: Fever

Adverse reactions at non-inoculated sites (systemic) : headache, fatigue/ asthenia, nausea, vomiting, diarrhea, muscle pain, cough, acute allergic reactions, mental disorders (specific symptoms)

Vital signs and physical examination:

Vital signs (temperature, blood pressure) and physical examination (skin, cardiopulmonary auscultation) should be checked before 2nd pre-vaccination, 3rd pre-vaccination (If applicable) and screening period.

ADE/VED risk monitoring: After vaccination (at least 1 dose of experimental vaccine) (at each visit), remind the subject of fever and/or respiratory symptoms (such as dyspnea, sore throat, etc.) during the study), contact the investigator in time when

	COVID-19 is suspected or confirmed. If the subject is suspected of being infected or confirmed to be infected with SARS-CoV-2 during the trial, he/she must go to the local designated hospital for hospitalization and treatment. For confirmed cases, detailed individual investigations are required, and for severe or dead cases, special investigations must be continued to analyze whether there is ADE/VED phenomenon. **Security data monitoring**: Set up DSMB to monitor the safety data during the study period. 1) DSMB is composed of 3 experts (1 each for clinical medicine, epidemiology and statistics). 2) DSMB is responsible for monitoring the safety data during the study period. 3) The safety data of all subjects in the 3-dose group after 30 days after full vaccination was reviewed by DSMB experts. 4) If a suspension/termination criteria event occurs, the DSMB will convene an emergency meeting to evaluate whether to terminate the trial, and promptly report to the ethics committee and the National Drug Evaluation Department. 5) In the case of severe or dead cases after the COVID-19 infection that occurred during the trial, the DSMB will convene an emergency meeting to conduct safety assessments and organize special investigations. If the results suggest the presence of ADE/VED phenomenon, it will be immediately reported to the ethics committee and the National Drug Evaluation Department. **Immunogenicity test：** Humoral immunity：Blood samples (8ml) will be collected before each dose of vaccination, 14 days after each dose, 1 month, 6 months and 12 months after the entire inoculation for detection of SARS-COV-2 neutralizing antibody, S protein binding antibody (IgG) and RBD protein binding antibody (IgG). Cell-mediated immunity：There are 72 subjects in each immunization program group and a total of 144 subjects (24 cases of low dose in 2-dose group, 24 cases of high dose in 2-dose group, 24 cases of placebo in 2-dose group; 24 cases of low dose in 3-dose group, 24 cases of high dose in 3-dose group, 24 cases of placebo in 3-dose group). Blood samples (30ml) will be collected before the first dose of vaccination, on the 4th

	day and 12 months after the full course of immunization for detection of cytokine IL-2, IL-4, IL-5, IL-6 and IFN-γ .
Inclusion criteria	1) Persons between 18 and 59 years of age (including 18 and 59 years of age) with full civil capacity； 2) The volunteers themselves agree to participate in the study, and sign an informed consent form, and can provide valid identification; understand and comply with the requirements of the trial protocol; 3) Axillary temperature < 37.3℃; 4) Female subjects of childbearing age agree to take effective contraceptive measures during the study.
Exclusion criteria for first dose inoculation	1)The population vital signs and physical examination results specified in the protocol are clinically meaningful judged by clinicians； 2)A history of severe allergies to any component of the test vaccine, including aluminum preparations, such as: anaphylactic shock, allergic laryngeal edema, allergic purpura, thrombocytopenic purpura, local allergic necrosis (Arthus reaction), dyspnea, blood vessels Neuroedema, etc.; or any previous history of severe adverse reactions following vaccine or drug use. 3)A history of SARS and SARS-COV-2 (Satisfy any of the following: ① A history of SARS and SARS-CoV-2 infection or disease history; ② During this SARS-CoV-2 epidemic, there was a history of contact with confirmed/suspected patients of the COVID-19); 4)Have taken antipyretics or painkillers within 24 hours before the first dose of vaccination 5)Inoculate subunit vaccine and inactivated vaccine within 14 days before the first dose of vaccination, and inoculate live attenuated vaccine within 30 days; 6)volunteer suffering from the following diseases: ① Patients with acute febrile illness； ② Suffering from digestive system diseases (such as diarrhea, abdominal pain, vomiting, etc.) in the past 7 days;

	③ Suffering from congenital malformations or developmental disorders, genetic defects, severe malnutrition, etc.; ④ History of congenital or acquired immunodeficiency, autoimmune diseases, or treatment with immunomodulators within 6 months (such as hormones, monoclonal antibodies, thymosin, and interferon, etc.), except for topical medication (such as ointment, eye drops, inhalation or nasal spray) ⑤ volunteer who have been diagnosed with infectious diseases, such as: tuberculosis, viral hepatitis patients and/or human immunodeficiency virus HIV antibody positive or syphilis specific antibody positive ⑥ Have a neurological disease or neurodevelopmental dysplasia (such as migraine, epilepsy, stroke, seizure in the last three years, encephalopathy, focal neurological deficits, Guillain-Barre syndrome, encephalomyelitis or transverse myelitis); a history or family history of mental illness; ⑦ Functional aspleen, as well as any cause of aspleen or splenectomy; ⑧ Suffering from serious chronic diseases or the disease is in the advanced stage and cannot be controlled smoothly, such as diabetes, thyroid disease; ⑨ Severe liver and kidney disease; the respiratory diseases that currently require daily medication (such as chronic obstructive pulmonary disease [COPD], asthma) or any treatment for exacerbations of respiratory disease within the last 5 years (for example, asthma exacerbations); a history of severe cardiovascular disease (such as congestive heart failure, cardiomyopathy, ischemic heart disease, arrhythmia, conduction block, myocardial infarction, pulmonary heart disease) or myocarditis or pericarditis; ⑩ A history of thrombocytopenia, any coagulation dysfunction or anticoagulant treatment, or have obvious bleeding; ⑪ The cancer patients； 7)Have received blood or blood-related products, including immunoglobulin, within 3 months; or plan to use it during the research period； 8)Women in lactation or pregnancy (positive urine pregnancy test test)； 9)Using any research or unregistered products (drugs, vaccines, biological products or

	devices) other than research products within 3 months, or planning to use them during the research period； 10)The researchers believe that any disease or condition in the subject may put the subject at an unacceptable risk; the subject cannot meet the requirements of the protocol; the situation that interferes with the assessment of the vaccine response.
Withdraw criteria	The researcher decided to withdraw: 1) Adverse events or concomitant events that cannot continue the trial occur; 2) Participating in other clinical trials before the end of this clinical trial； 3) Other situations where the investigator believes that the subject is not suitable for continuing to participate in this clinical study； Subject-determined withdrawal: The subject has the right to withdraw from the study at any stage of the study.
Study endpoint	**Immunogenicity endpoints:** Primary endpoint: Positive conversion rate of neutralizing antibody 30 days after full inoculation in negative population before immunization; Secondary endpoint: ① The GMTs of neutralizing antibodies and positive rate 14 days after the first dose of vaccination in negative population before immunization; ② The GMTs of neutralizing antibodies and positive rate/positive conversion rate 14 days after full inoculation in negative population before immunization; ③ The GMTs of neutralizing antibodies 30 days after full inoculation in negative population before immunization； ④ The GMTs of neutralizing antibodies both 6 months and 12 months after full inoculation; ⑤ The GMIs of neutralizing antibodies and positive rate both 6 months and 12 months after full inoculation; ⑥ The levels of IL-2, IL-4, IL-5, IL-6 and IFN- γ on the 4th day and 12 months after

<table>
<tr><td></td><td>full inoculation.

Safety endpoint:

① Analysis of adverse events from fist dose of vaccination to 30 days after full immunization: Incidence of adverse events; Incidence of adverse reactions; The incidence of grade 3 or above adverse events; The incidence of grade 3 or above adverse reactions; The incidence of adverse events leading to withdrawal; The incidence of adverse reactions leading to withdrawal.

② Analysis of serious adverse events on day 0 of vaccination to 12 months after full immunization: Incidence of serious adverse events; Incidence of serious adverse events associated with experimental vaccines.</td></tr>
<tr><td>Sample size consideration</td><td>A total of 900 subjects will be enrolled in the trial, including 150 cases in the low-dose group with 2 doses, 150 cases in the high-dose group with 2 doses, 150 cases in the placebo-controlled group with 2 doses, 150 cases in the low-dose group with 3 doses, and 150 cases in the high dose group with 3 doses, and 150 cases in the placebo control group with 3 doses. It is assumed that the positive rate of neutralizing antibody reached 80% in the vaccine group and 30% in the placebo group, and the test level was unilateral α =0.025. With this sample size, the difference between vaccine and placebo can be found with 99.99% confidence.</td></tr>
<tr><td>Criteria for suspension or termination of tests</td><td>This study or any other study has obtained new data about the research vaccine, the administrative department, the sponsor, the investigator, and/or the IRB suggest that the trial should be suspended/terminated;

Suspended test criteria:

In any of the following situations, the trial needs to be suspended and immediately reported to the ethics committee, provincial and national drug administration departments; and DSMB experts will be urgently convened to conduct a safety demonstration analysis to determine whether to continue the trial.

<table><tr><td>The event that caused the suspension of the test</td><td>Number of cases/ %</td></tr></table></td></tr>
</table>

<table>
<tr><td></td><td>
<table>
<tr><td>Vaccine-related deaths or serious life-threatening adverse reactions occurred during the study period</td><td>⩾1 case</td></tr>
<tr><td>Severe or death cases after novel coronavirus infection occurred during the study period</td><td>⩾1 case</td></tr>
<tr><td>Adverse events ⩾ Grade 3 and lasting 48 hours after any dose of vaccine</td><td>> 15% Number of vaccinate persons</td></tr>
</table>
Test termination criteria:
In the following cases, the trial shall be terminated and immediately reported to the DSMB, the ethics committee, provincial and national drug administration departments.
<table>
<tr><td>An event that results in the termination of a test</td><td>number cases %</td></tr>
<tr><td>Adverse events ⩾ Grade 3 and lasting 48 hours after any dose of vaccine</td><td>>30% Number vaccinated perso</td></tr>
</table>
</td></tr>
<tr><td>Related definitions</td><td>1) "Month" in the visit: defined as "30 days";
2) Fertile woman: refer to women who are in the specific period from the development of female reproductive organs (menarche) to the decline of ovarian function (menopause);
3) 18-59 years old: 18 years old on the day of enrollment (i.e. the day when 18 years old), and the maximum is under 60 years old (i.e. the day before 60 years old);
4) Antibody positive rate: antibody titer before immunity < cut-off value, antibody titer after immunity ⩾ cut-off value or antibody titer before immunity > cut-off value, and percentage of subjects whose antibody titer increased by 4 times or more after immunity.</td></tr>
<tr><td>Study duration</td><td>16 months.</td></tr>
<tr><td>Statistical analysis</td><td>In the basic immunization stage, the safety database will be locked after all subjects completed the full immunization for 1 month, and the safety and immunogenicity data will be analyzed for the first time for 1 month after the full immunization in the basic</td></tr>
</table>

strategy	immunization stage. The safety data (especially serious adverse events) and immune persistence data will be updated and analyzed 6 months after full vaccination. After the immune persistence phase is completed, the immune persistence data for 12 months after the full vaccination will be updated and analyzed.

Diagram of visit process (2-dose group)

Visit	V1	V2	V3	V4	V5#	V6	V7	V8	V9	V10
Visit time (day)[1]	**D0**	**V1+8**	**V1+14**	**V1+30**	**V4+4**	**V4+8**	**V4+14**	**V4+30**	**V4+180**	**V4+360**
Window period (day)	/	/	±1	+7	+3	/	±1	+7	+30	+30
Informed consent	●									
Demographic information	●									
Height and weight	●									
Medical history inquiry	●			●						
Vital signs (body temperature, and blood pressure)	●			●						
Physical examination (skin, the auscultation of the heart and lung)	●			●						
Urine pregnancy test test (5.0~10.0mL urine)	●			●						
Inclusion Criteria & Exclusion Criteria, Delayed vaccination standards / Termination criteria	●			●						
Assign study number	●									

Visit	**V1**	**V2**	**V3**	**V4**	**V5#**	**V6**	**V7**	**V8**	**V9**	**V10**
Visit time (day)[1]	**D0**	**V1+8**	**V1+14**	**V1+30**	**V4+4**	**V4+8**	**V4+14**	**V4+30**	**V4+180**	**V4+360**
Window period (day)	/	/	±1	+7	+3	/	±1	+7	+30	+30
SARS-CoV-2 real-time fluorescent RT-PCR nucleic acid detection	●									
Detecting IgM and IgG antibody of SARS-CoV-2	●									
Blood sampling for humoral immunity (8mL)[2]	●		●	●			●	●	●	●
Blood sampling for cellular immunity（30ml）	●				●					●
Vaccination	●			●						
Observe for 30 minutes after vaccination	●			●						
Distribute thermometers, measuring rulers, diary cards and train[3]	●			●						
Recycle diary cards and issue contact cards		●				●				

Visit	**V1**	**V2**	**V3**	**V4**	**V5#**	**V6**	**V7**	**V8**	**V9**	**V10**
Visit time (day)[1]	**D0**	**V1+8**	**V1+14**	**V1+30**	**V4+4**	**V4+8**	**V4+14**	**V4+30**	**V4+180**	**V4+360**
Window period (day)	/	/	±1	+7	+3	/	±1	+7	+30	+30
Recycle contact cards				●				●		
Report serious adverse events	●	●	●	●	●	●	●	●	●	●
ADE/VED Risk monitoring（Report suspected or confirmed cases of COVID-19）[4]	●	●	●	●	●	●	●	●	●	●
Concomitant medication [5]	●	●	●	●	●	●	●	●	●	●

Subjects who only participate in the humoral immunity assessment will not conduct V5

1. The day of each dose of vaccination is not counted in the visit time (For example: if the first dose of vaccination (V2) is inoculated on April 1, the diary card (V2+8) should be returned on April 9);

2. V2 and V4 need to complete immunogenic blood sampling before vaccination;

3. Distribute thermometers, measuring rulers and diary cards at V2, and distribute diary cards at V4;

4. If the subject is suspected or confirmed to be infected with SARS-CoV-2 during the clinical trial, he must go to the local designated hospital for hospitalization for diagnosis and treatment;

5. Only the drugs used for the treatment of SAE and pregnancy complications will be collected for the combined medication after 30 days to 12 months of full immunization.

Diagram of visit process (3-dose group)

Visit	V1	V2	V3	V4	V5	V6	V7	V8#	V9	V10	V11	V12	V13
Visit time (day)[1]	**D0**	**V1+8**	**V1+14**	**V1+30**	**V4+8**	**V4 +14**	**V4+30**	**V7+4**	**V7+8**	**V7+14**	**V7+30**	**V7+180**	**V7+360**
Window period (day)	/	/	±1	+7	/	±1	+7	+3	/	±1	+7	+30	+30
Informed consent	●												
Demographic information	●												
Height and weight	●												
Medical history inquiry	●			●			●						
Vital signs (body temperature, and blood pressure)	●			●			●						
Physical examination (skin, the auscultation of the heart and lung)	●			●			●						
Urine pregnancy test test (5.0~10.0mL urine)	●			●			●						
Inclusion Criteria & Exclusion Criteria, Delayed vaccination standards / Termination criteria	●			●			●						

Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8#**	**V9**	**V10**	**V11**	**V12**	**V13**
Visit time (day)[1]	**D0**	**V1+8**	**V1+14**	**V1+30**	**V4+8**	**V4 +14**	**V4+30**	**V7+4**	**V7+8**	**V7+14**	**V7+30**	**V7+180**	**V7+360**
Window period (day)	/	/	±1	+7	/	±1	+7	+3	/	±1	+7	+30	+30
Assignment study number	●												
SARS-CoV-2 real-time fluorescent RT-PCR nucleic acid detection	●												
Detecting IgM and IgG antibody of SARS-CoV-2	●												
Blood sampling for humoral immunity (8mL)[2]	●		●	●		●	●			●	●	●	●
Blood sampling for cellular immunity（30ml）	●							●					●
Vaccination	●			●			●						
Observe for 30 minutes after vaccination	●			●			●						
Distribut thermometers, measuring rulers, diary cards and train[3]	●			●			●						
Recycle diary cards and issue		●			●				●				

Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8#**	**V9**	**V10**	**V11**	**V12**	**V13**
Visit time (day)[1]	**D0**	**V1+8**	**V1+14**	**V1+30**	**V4+8**	**V4 +14**	**V4+30**	**V7+4**	**V7+8**	**V7+14**	**V7+30**	**V7+180**	**V7+360**
Window period (day)	/	/	±1	+7	/	±1	+7	+3	/	±1	+7	+30	+30
contact cards													
Recycle contact cards				●			●				●		
Report serious adverse events	●	●	●	●	●	●	●	●	●	●	●	●	●
DE/VED Risk monitoring （Report suspected or confirmed cases of COVID-19）[4]	●	●	●	●	●	●	●	●	●	●	●	●	●
Concomitant medication [5]	●	●	●	●	●	●	●	●	●	●	●	●	●

Subjects who only participate in the humoral immunity assessment will not conduct V8

1. The day of each dose of vaccination is not counted in the visit time (For example: if the first dose of vaccination (V2) is inoculated on April 1, the diary card (V2+8) should be returned on April 9);

2. V2, V4, V7 need to complete immunogenic blood sampling before vaccination;

3. Distribute thermometers, measuring rulers and diary cards at V2, and distribute diary cards at V4 and V7;

4. If the subject is suspected or confirmed to be infected with SARS-CoV-2 during the clinical trial, he must go to the local designated hospital for hospitalization for diagnosis and treatment;

5. Only the drugs used for the treatment of SAE and pregnancy complications will be collected for the combined medication after 30 days to 12 months

Visit	V1	V2	V3	V4	V5	V6	V7	V8#	V9	V10	V11	V12	V13
Visit time (day)[1]	**D0**	**V1+8**	**V1+14**	**V1+30**	**V4+8**	**V4 +14**	**V4+30**	**V7+4**	**V7+8**	**V7+14**	**V7+30**	**V7+180**	**V7+360**
Window period (day)	/	/	±1	+7	/	±1	+7	+3	/	±1	+7	+30	+30
of full immunization.													

A Phase Ⅱ, Randomized, Double-Blind, Placebo-Controlled Clinical Trial to Evaluate the Immunogenicity and Safety of the Recombinant Novel Coronavirus Vaccine (CHO cells) with Different Doses and Different Immune Procedures in Healthy volunteers aged 18-59 Years

1. Introduction

The recombinant novel coronavirus vaccine (CHO cell) developed by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. belongs to the first category of preventive biological products and is an innovative vaccine that has not been marketed at home and abroad. It is proposed to be applicable to the prevention of respiratory diseases caused by the novel coronavirus infection. With the urgent needs of epidemic prevention and control on COVID-19, the clinical trials were approved on June 19th, 2020 by NMPA in accordance with "*The Drug Administration Law of the PRC*" and " *Provisions for Drug Registration* " (drug clinical trial approval documents: 2020L00023, 2020L00024).

The sponsor, Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. commissioned the Hunan Provincial Center for Disease Control and Prevention to conduct a phase Ⅱ clinical trial to evaluate the effects of novel Coronavirus vaccine (CHO cell) with different doses and immunization procedures on immunogenicity in healthy volunteers aged from 18 to 59. According to the "*Provisions for Drug Registration* ", "*Good Clinical Practice* ", "*Guiding Principles for Quality Management of Vaccine Clinical Trials*" and other relevant regulations issued by NMPA, combined with the early safety results of phase I clinical trials, a phase Ⅱ clinical trial protocol of recombinant novel coronavirus vaccine (CHO cell) was formulated.

2. Background and rationale

2.1 Diseases and pathogens

2.1.1 Disease background

Since December 2019, a number of novel coronavirus-infected pneumonia cases have been reported globally. As of May 28th,2020, the cumulative number of confirmed cases in China reached 84,547, and the cumulative number of deaths was 4,645. A total of confirmed cases abroad was 5,712,822, and 353,048 deaths. On January 31st , 2020, the World Health

Organization declared the outbreak of the novel coronavirus as a global public health emergency. On February 11th, 2020, the World Health Organization announced that the name of novel coronavirus-infected pneumonia as "COVID-19".

At present, the visible source of infection is mainly patients infected with the novel coronavirus, and asymptomatic infections can also become the source of infection. The main transmission routes are droplet transmission and contact transmission. There is possibility of aerosol transmission if exposed to high concentrations of aerosols for a long time in a relatively closed environment. Human is generally susceptible. Based on current epidemiological investigations, the incubation period of the disease ranges from 1 to 14 days, mostly 3 to 7 days. The main symptoms of patients are fever, fatigue, and dry cough. A few patients are accompanied with nasal congestion, runny nose, sore throat and diarrhea. Severe patients often develop dyspnea and (or) hypoxemia after one week from onset of the disease. In severe cases, they rapidly progress to acute respiratory distress syndrome, septic shock, incorrigible metabolic acidosis and coagulation dysfunction and multiple organ failure, etc. According to the status of the currently cases in hospitalization, most patients had a good prognosis, and a few patients were critically ill or even died. The prognosis of the elderly and those with chronic underlying diseases is poor. Symptoms in children are relatively mild.

2.1.2 Pathogen background

Novel Coronavirus is a coronavirus belonging to the beta genus, with an envelope of a circular or elliptic particle, often pleomorphic, of which diameter is 60-140 nM. Its genetic characteristics are significantly different from SARS-COV (severe acute respiratory syndrome coronavirus) and MERSR-COV (Middle East Respiratory syndrome coronavirus).The homology with BAT SARS-like coronavirus (BAT-SL-COvZC45) is over 85%. In vitro isolation and culture, SARS-CoV-2 (Novel Coronavirus) could be found in human respiratory epithelial cells within 96 hours, while the isolation and culture of VERO E6 (African green monkey kidney cell line) and HUH-7 (human liver cancer cell line) took about 6 days. The understanding of the physicochemical properties of coronavirus mainly comes from the studies on SARS-CoV and MERS-CoV. Virus is sensitive to UV and heat, 56℃ for 30 minutes, ethyl ether, 75% ethanol, chlorine-containing disinfectant, peracetic acid and chloroform lipid solvents which can effectively inactivate the virus, but chlorhexidine can not effectively inactivate the virus.

2.2 Novel coronavirus vaccine background

On February 11th, 2020, the WHO officially named the novel coronavirus pneumonia as

"COVID-19". On the same day, the International Virus Classification Committee also announced that the novel type of coronavirus that caused the disease was officially named "SARS-CoV-2". In the face of the surging epidemic, researchers at home and abroad rushed to invest in vaccine research, and began a race against the virus. On 26th-27th, January, 2020, the Chinese Center for Disease Control and Prevention and the Zhejiang Provincial Center for Disease Control and Prevention successively isolated the SARS-CoV-2 virus strain, laying the foundation for vaccine research. Like SARS-CoV and MERS-CoV, the S protein of SARS-CoV-2 and the receptor binding domain (RBD) contained in it are still the main targets of vaccine development. Globally, with the advocacy and funding of the Epidemic Prevention Innovation Alliance, vaccine development projects for SARS-CoV-2 have also been announced.

In terms of vaccine types, the Faculty of Medicine of the University of Hong Kong in China and the Pasteur Institute in France will respectively improve the existing influenza vaccine and measles vaccine by adding the expression sequence of the SARS-CoV-2 S protein to shorten the development cycle; the United States Johnson & Johnson Pharmaceuticals, Wuhan Bowo Biotech and GeoVax Labs will use their adenovirus and modified poxvirus platforms to develop a SARS-CoV-2 viral vector vaccine; the University of Queensland in Australia will use its unique "molecular tweezers" vaccine platform. The SARS-CoV-2 S protein synthesized in vitro is "clamped" into a natural polymerized state, which is convenient to induce the production of neutralizing antibodies in vivo. This research is also assisted by the powerful "adjuvant platform technology" of GlaxoSmithKline to further enhance the immune response stimulated by this subunit vaccine in the body; Beijing Aidiweixin Biology will cooperate with Inovio of the United States to develop a DNA vaccine "INO-4800" containing the SARS-CoV-2 S protein sequence. At the same time, more companies and scientific research institutions are focusing on the research and development of novel mRNA vaccines. This latest technology uses mRNA encoding viral antigens as vaccines, which are translated into viral proteins by direct injection or delivery into the body. It induces cellular and humoral immune response, and has the characteristics of low immunogenicity, short production process and fast research and development speed. As the sequence of SARS-CoV-2 is quickly deciphered and announced, researchers can quickly screen antigens, design and synthesize multiple potential mRNA vaccines for SARSCoV-2. At present, mRNA vaccine research and development companies such as Moderna in the United States and CureVac in Germany, as well as domestic Shanghai Si Microbial Technology Co., Ltd. and Guangzhou Guanhao Biotechnology Co., Ltd. have announced that they will cooperate with scientific research institutes or independently develop SARS-CoV-2 mRNA vaccines.

2.3 Principles of clinical trial design

2.3.1 Principles of experimental design and control selection

The recombinant novel coronavirus vaccine (CHO cells) developed by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. belongs to Class 1 preventive biological products, and is an innovative vaccine that has not been marketed at home or abroad.

COVID-19 is a susceptible diseases to all ages. Based on the current epidemiological investigation, the incubation period rages from 1 to 14 days. Judging from the status of the currently cases, the prognosis of the elderly and those with chronic underlying diseases is poor, and the symptoms of children are relatively mild. According to data from the Chinese Center for Disease Control and Prevention, most cases (77.8%) are between 30-69 years old, and the attack rate for volunteers aged 18 and under is relatively low (accounting for 2.4% of all reported cases). Considering that≥60 years old is classified as the elderly in China, this clinical trial sets the age of the target population from 18 to 59 , and observes the safety of the first dose of vaccination to 12 months after the full vaccination.

This trial adopts a single-center, blind, randomized, placebo-controlled clinical trial design.

For specific protocol design and sample size calculation, please refer to the chapters "7 Research Design" and "12.3 Sample Size Consideration".

2.3.2 Selection basis for dosage and immunization program

According to the analysis of the dose-effect relationship of the antigen dose, it is determined that the vaccine includes two doses: each dose contains 25 μg / 0.5 mL / bottle (low dose) and each dose contains 50 μg / 0.5 mL / bottle (high dose).

2.4 Subject's benefits/potential risks

2.4.1 The known potential risks

Participating in this study may prevent the respiratory disease (COVID-19) caused by the novel coronavirus infection, and like many other vaccines, its immune effect needs to be evaluated in clinical trials. At the same time, some volunteers in this study were administered a placebo, there will be no protection against the novel coronavirus infection, therefore, the disease may be caused by natural infection with the novel coronavirus during the observation period.

At the same time, in some cases, antibodies play a role in enhancing virus infection during viral infection. They assist the virus to enter target cells and increase the infection rate, which is called Antibody dependence enhancement (ADE). In the last century, in clinical trials of

respiratory syncytial virus vaccines, it was found that 80% of subjects were hospitalized for Vaccine enhanced disease (VED), and two of the subjects eventually died. Since the SARS vaccine preclinical animal test (rhesus monkey) observed that the pathological damage score of the lung organs after vaccination was higher than that of the placebo group, it is necessary to pay close attention to whether the subjects have fever and / or respiratory symptoms, suspected or confirmed cases of COVID-19, etc. If the subject is suspected or confirmed to be infected with SARS-CoV-2 during the trial period, he must go to the local designated hospital for hospitalization.

The potential risks of the trial vaccine are limited to any common adverse reactions of the vaccine, such as mild pain at the injection site and occasional mild to moderate flushing, swelling and induration. Fever and anorexia may also occur, but it is expected to be mild. Generally, it will relieve itself and disappear without treatment; individual subjects may have strong reactions (such as high fever, allergic reactions, etc.), and the researcher will closely observe and treat according to the symptoms.

The research process requires blood sampling. After the blood sampling, pain or ecchymosis may occur.

2.4.2 The known potential benefits

Subjects may gain potential protection from the test vaccine, that is, to prevent the respiratory disease COVID-19 caused by the novel coronavirus infection. By participating in this registration trial, the subjects will contribute to the launch of the novel coronavirus vaccine and benefit a wider population.

2.4.3 Assessment of potential risks and benefits

At present, there is no novel coronavirus vaccine on the market at home and abroad. Subjects may gain potential benefits from the trial vaccine, that is, to prevent respiratory diseases caused by novel coronavirus infection.

The potential risks of the experimental vaccine are limited to any common adverse reactions of the vaccine, such as pain, redness, swelling and induration at the injection site, as well as fever, irritation or suppression, and loss of appetite, but it is expected to be mild. During the study, researchers will closely monitor the side effects of the vaccine. If the subject experiences any side effects or discomfort, the investigator should be notified in time. If the investigator or subject believes that these side effects cannot be tolerated, the follow-up trial vaccination may be stopped or terminated, and the safety will be closely followed.

3. Product characteristics and preclinical research/laboratory evaluation

3.1 Product features

This product is made by purifying the receptor binding region of the novel coronavirus spike glycoprotein (recombinant protein NCP-RBD) expressed by recombinant CHO cells and adding aluminum hydroxide as adjuvant. It is used to prevent the respiratory diseases caused by novel coronavirus infection. It is a milk-white suspension, which can be layered by precipitation and easy to shake off.

3.2 Product preparation and verification

The recombinant novel coronavirus vaccine (CHO cell) belongs to the first class of preventive biological products, and there is no reference substance and reference substance used in the quality study of this project. According to the structure of the project and the characteristics of the process, in accordance with the ICH (International Coordination Conference on Drug Registration Technology for Human Use) guidelines and the National Medical Products Administration's "*Provisions for Drug Registration* " and other requirements for the preparation of the quality of preventive biological products, the primary liquids were prepared. Detailed quality research has been conducted on the intermediate products, primary liquids, semi-finished products and finished products, and corresponding quality standards have been formulated.

This product is prepared by pilot production process and in accordance with GMP requirements and corresponding conditions. The repeatability between batches is good and the quality is stable. Product quality verification is carried out in accordance with the "*Chinese Biological Products Regulations*", and the quality standards have been reviewed and qualified by the China Food and Drug Control Institute (referred to as the China Inspection Institute). The unit price of the stock solution and the finished product have reached the established quality standards, the protein purity is higher than 95.0%, the bacterial endotoxin is less than 10EU/dose, and the vaccine potency meets the batch standard. There are no potentially toxic substances to the human body.

3.2.1 Stability test

In accordance with the requirements of the drug stability research guidelines, preliminary stability studies have been conducted on 6 consecutive batches of recombinant novel coronavirus vaccines (CHO cells), including 3 batches of 50 μg / 0.5 ml / bottle. Remove the outer packaging of the vaccine and place it in a suitable open container, and conduct mandatory stability tests (shock test, light test and high temperature test), accelerated stability test and long-term stability test respectively.

The shock test is to place the qualified vaccine in a shaking shaker at 2~8° C for 28 days. At present, except for the efficacy test on the 28th day, the test has been completed at other time points and all comply with the regulations. The light test is to place the qualified vaccine at 2~8 °C and the illuminance of 4500lx±500lx for 28 days. So far, the test has been completed for 7, 14, and 21 days, and they all meet the requirements. The high temperature test is to place the qualified vaccine under 37±2℃ constant temperature for 28 days, and the compulsory stability test is used to judge the stability of the vaccine deviating from the normal storage conditions. At present, the efficacy test on the 28th day has not been completed, the testing at other time points has been completed and all comply with the regulations. In addition, the qualified vaccine is subjected to an accelerated stability test for 6 consecutive months under 25 ± 2 ℃ environmental conditions to investigate the stability test and a long-term stability test for 2.5 years under 2-8 ℃ environment to investigate the stability test. At present, the accelerated test has completed a one-month inspection and is in compliance with regulations.

3.2.2 Immunogenicity

At present, through research on Balb/c mice, SD rats and Cynomolgus monkey, blood was collected at different immune stages for IgG antibody level detection, pseudovirus neutralizing antibody level detection, and true virus neutralizing antibody level detection, and the spleen was taken for ELISpot Tests. The research results show that the vaccine can reach a high antibody titer after two immunizations, and has a certain neutralizing effect on both pseudovirus and true virus, and can stimulate the secretion of antigen-specific cytokines by spleen cells, indicating that the vaccine has good immunogenicity.

3.2.3 Safety evaluation

The safety evaluation research of this vaccine includes rat single-dose toxicity test, rabbit intramuscular injection stimulation test, guinea pig systemic active allergy test, monkey 4-week repeated administration toxicity dose exploration test (including immunity and safety pharmacological indicators), 4 weeks repeated administration toxicity test in rats and 8 weeks repeated administration toxicity test in monkeys (with immunogenicity, immunotoxicity, safety pharmacology test). So far, we have completed the rat single-dose toxicity test, rabbit intramuscular injection stimulation test, guinea pig systemic active allergy test, and monkey 4-week repeated administration toxicity dose exploration test (including immunity and safety pharmacological indicators). The interim report of the 4-week repeated administration toxicity test in rats has been completed. From the current safety evaluation results, the

recombinant novel coronavirus vaccine (CHO cells) is safe and reliable, it has good immunogenicity, and can support the development of clinical trials. In the later stage, other trial data will be submitted on a rolling basis to fully evaluate the safety of the vaccine.

4. Research purpose

4.1 Main purpose

To evaluate the immunogenicity and safety of the novel coronavirus vaccine (CHO cells) with different doses and different immune procedures in healthy volunteers aged 18-59 years

4.2 Secondary purpose

Further explore the immune durability of the novel coronavirus vaccine (CHO cells) with different doses and different immune procedures

5. Clinical trial institution and site

Clinical trial institution: Hunan Provincial Center for Disease Control and Prevention;

Clinical trial site: Xiangtan Center for Disease Control and Prevention;

Refer to the appendix for the introduction of clinical trial institutions and trial sites.

6. Study endpoint

6.1 Endpoint of immunogenicity

- Primary evaluation endpoint:

Positive conversion rate of neutralizing antibody 30 days after full inoculation in negative population before immunization.

- Secondary evaluation endpoint:

① The GMTs of neutralizing antibodies and positive rate 14 days after the first dose of vaccination in negative population before immunization;

② The GMTs of neutralizing antibodies and positive rate/positive conversion rate 14 days after full inoculation in negative population before immunization;

③ The GMTs of neutralizing antibodies 30 days after full inoculation in negative population before immunization；

④ The GMTs of neutralizing antibodies both 6 months and 12 months after full inoculation.

⑤ The GMIs of neutralizing antibodies and positive rate both 6 months and 12 months after full inoculation.

⑥ The levels of IL-2, IL-4, IL-5, IL-6 and IFN-γ on the 4th day and 12 months after full

inoculation.

6.2 Safety endpoint

- Analysis of adverse events from the first dose of vaccination to 30 days after full immunization: incidence of adverse events; Incidence of adverse reactions; The incidence of grade 3 or above adverse events; The incidence of grade 3 or above adverse reactions; The incidence of adverse events leading to withdrawal; The incidence of adverse reactions leading to withdrawal.
- Analysis of serious adverse events from the day of vaccination to 12 months after full immunization: incidence of serious adverse events; Incidence of serious adverse events associated with experimental vaccines.

6.3 Related definitions

1) "Month" in the visit: defined as "30 days";

2) Fertile woman: refer to women who are in the specific period from the development of female reproductive organs (menarche) to the decline of ovarian function (menopause);

3) 18-59 years old: 18 years old on the day of enrollment (i.e. 18 years old), less than 60 years old (i.e. the day before 60 years old);

4) Antibody positive conversion rate: antibody titer before immunity < cut-off value, antibody titer after immunity ≥ cut-off value or antibody titer before immunity > cut-off value, and percentage of subjects whose antibody titer increased by 4 times or more after immunity.

7. Study Design

7.1 Overall design

This trial uses a single-center, randomized, blinded, placebo-controlled trial design to evaluate the immunogenicity and safety of recombinant novel coronavirus vaccines (CHO cells) with different doses and different immune procedures in healthy volunteers aged 18 to 59 years old.

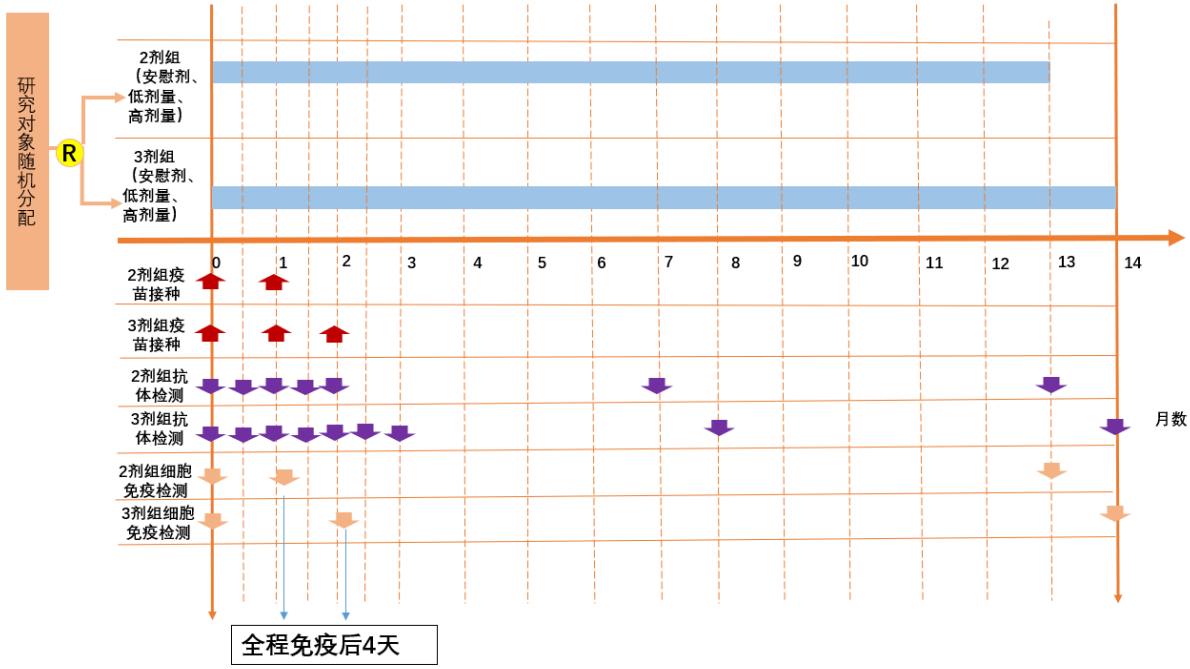


Fig. 1 An overview of experimental design

7.1.1 Sample size and trial grouping

A total of 900 subjects will be enrolled in this study, and they will be randomly assigned to the 2 doses of low-dose experimental vaccine group, 2 doses of high-dose experimental vaccine group, and 2 doses of placebo group , 3 doses of low-dose experimental vaccine group, 3 doses of high-dose experimental vaccine group, 3 doses of placebo group at a ratio of 1:1:1:1:1:1, and The sample size of each group is 150 cases (For detailed sample size estimation, refer to "12.3 Sample Size Consideration").

7.1.2 Vaccination and follow-up

Subjects in the 2-dose group are injected with 2 doses of trial vaccine or placebo into the deltoid muscle of the upper arm according to the 0, 1-month vaccination procedure. Subjects who only participate in the humoral immunity assessment need to complete 9 scheduled on-site visits (not attending V5), and subjects participating in the humoral and cellular immunity assessment are required to complete 10 scheduled on-site visits. See "Visit Flow Chart (2-dose group)" for details.

Subjects in the 3-dose group are injected with 3 doses of trial vaccine or placebo into the deltoid muscle of the upper arm according to the 0, 1, 2-month vaccination procedure. Subjects who only participate in the humoral immunity assessment need to complete 12 scheduled on-site visits (not attending V8), and subjects participating in the humoral and cellular immunity assessment are required to complete 13 scheduled on-site visits. See "Visit Flow Chart (3-dose

group)" for details. After the first two doses of the 3-dose group, the third dose of vaccination can only be carried out after the safety data is evaluated and approved by the national drug review department. If the national drug review department does not allow the third dose of vaccination, subjects will be referred to the 2-dose group for follow-up visits.

7.1.3 Immunogenicity observation

Humoral immunity： Blood samples (8 ml) will be collected before each dose of vaccination, 14 days after each dose of vaccination, 1 month, 6 months and 12 months after the entire inoculation, and tested for SARS-CoV-2 neutralizing antibody, S protein binding antibody (IgG) and RBD protein binding antibody (IgG).

Cell-mediated immunity: There are 72 subjects in each immunization program group, a total of 144 subjects (24 cases of low dose in 2-dose group, 24 cases of high dose in 2-dose group, 24 cases of placebo in 2-dose group; 24 cases of low dose in 3-dose group, 24 cases of high dose in 3-dose group, 24 cases of placebo in 3-dose group). Blood samples (30 ml) will be collected before the first dose of vaccination, on the 4th day and 12 months after the full course of immunization, and tested for cytokine IL-2, IL-4, IL-5, IL-6 and IFN-γ detection.

7.1.4 Safety observation

- **Adverse event collection:**

All adverse events (AE) were collected 30 minutes after each dose of vaccination, 0-7 days (both recruited and non-recruited), and 8-30 days (non-recruited), and all serious adverse events (SAE) were collected from the first dose of vaccination to 12 months after the full dose of vaccination.

- **Vital signs and physical examination**

Vital signs (temperature, blood pressure) and physical examination (skin, cardiopulmonary auscultation) should be checked before each dose of vaccine and screening period.

- **ADE/VED risk monitoring**

After vaccination (at least 1 dose of experimental vaccine) (at each visit), remind the subject of fever and/or respiratory symptoms (such as dyspnea, sore throat, etc.) during the study), contact the investigator in time when COVID-19 is suspected or confirmed. If the subject is suspected of being infected or confirmed to be infected with SARS-CoV-2 during the trial, he must go to the local designated hospital for hospitalization and treatment. For

confirmed cases, detailed individual investigations are required, and for severe or dead cases, special investigations must be continued to analyze whether there is ADE/VED phenomenon.

7.2 Randomization and blinding

7.2.1 Randomization

Randomization: 900 numbers were randomized by a randomization statistician using SAS statistical software (version 9.4) by block group randomization. The subject ratio was 1:1:1:1:1:1 for the 2-dose low-dose group, 2-dose high-dose group, 2-dose placebo group, 3-dose low-dose group, 3-dose high-dose group, and 3-dose placebo group. The study numbers were 001-900. Investigators at trial site assign study numbers strictly according to the order of screening sequence of eligible subjects, and experimental vaccines were obtained and administered according to the numbers.

In this trial, 240 spare vaccines numbered B001-B240 were prepared and divided into low-dose, high-dose and placebo groups according to 1:1:1. SAS statistical software was used to generate a random assignment table for the alternate vaccine by a randomization statistician. When an alternate vaccine is needed for a study, the investigator logs into the alternate vaccine acquisition system to obtain the corresponding number of the alternate vaccine (the investigator only sees the study number, and the real groups of the trial vaccine and the alternate vaccine are imported into the system in advance, which are always blinded throughout the trial; when the number of alternate vaccines is insufficient, they can be added at any time).

The randomization statistician prepares a blind code for vaccine coding, which is divided into two copies, namely an original and a duplicate, one kept by the sponsor's staff and another kept by the principal investigator.

7.2.2 Blinding

This trial uses a blinding design. Randomization statisticians and other blinding staff will perform vaccine blinding, that is, paste the printed vaccine label on the designated position of each vaccine according to the blind code. The randomization statistician supervises the blinding of vaccines, and guides the blinding operators to label according to the blind code. After completing the blinding, the blind code should be sealed by the randomization statistician. The entire process of blind editing must be recorded. The blind editors are not

allowed to participate in other related work of this clinical trial. At the same time, they are not allowed to disclose the blind code to any personnel participating in this clinical trial.

7.2.3 Blindness maintenance

In this trial, a placebo was used as a control, and the packaging was blinded so that the test vaccine and the placebo could be identical in appearance. There are two types of vaccination procedures in this study. Due to the different vaccination procedures, it is not possible to be blinded between the vaccination procedures; therefore, the blinded operation is mainly for the test vaccine and placebo in the 2-dose and 3-dose groups respectively. After subjects are assigned a number, the investigator will be able to obtain the vaccination procedure by scratching the corresponding numbered vaccination procedure assignment scratch card.

7.2.4 Emergency unblinding

The randomization statistician prepares emergency letters while the blinding codes are performing. Each letter contains a random code for blinding, each of which corresponds to a vaccine number with feedback from the online blinding system on the true grouping of that vaccine number. Each random code represents a one-time chance to unblind. Only one vaccine number can be used for emergency unblinding, and then it will become invalid. Emergency letters are sent to the site with the blinded vaccine and kept by the site manager. All emergency envelopes are collected after completion of the trial, and the status of the emergency envelopes is checked for unblinding at the blinding review meeting.

In case of serious adverse events during the study, participants need to be rescued or received emergency treatment, the kind of vaccine need to be known. The investigator in charge at the test site, with oral or written authorization from the principal investigator, may decide whether or not to perform an individual emergency blinding, and may open the emergency letter to perform the emergency unblinding in accordance with the procedure.（Log in to the online emergency unblinding system with the random unblinding code in the letter and follow the instructions.）, and relevant records are made; this should subsequently be reported promptly (within 24 hours) to the principal investigator, sponsor, supervisor, ethics review committee and drug regulatory agency at the responsible institution. Subjects with that study number will terminate the trial for detachment, and the investigator will record the reason for termination in the case report form. Opened emergency letters should be kept in a safe place and returned to the sponsor at the end of the trial.

7.2.5 Unblinding requirements

This test uses a one-time unblinding method. After the subjects have completed the full vaccination and 1-month safety observation, the data files will be reviewed blindly and the database will be locked after it is deemed reliable and unblinded. This unblinding will list the treatment group to which each subject belongs. The results of the unblinding were submitted to a statistician for statistical analysis. The unblinding is performed by the principal investigator, who keeps a record of the unblinded subjects.

7.3 Study protocol revision

Any modification to this research protocol should be negotiated with the sponsor and approved. If an agreement is reached on the necessity of modification, the sponsor will make a written record, and the revised draft of the research protocol will replace the earlier version. All protocol revisions need to be submitted to the IRB, and important revisions (for example: revisions that affect the execution of the study and the safety of subjects) need IRB's approval. Modifications that do not affect the research design, research purpose, or the safety of the subjects are sent to the IRB for filing or rapid review (in accordance with the requirements of the IRB).

Except to eliminate the obvious immediate danger to the subjects, investigator have the rights to ensure that the changes shall not be implemented until the IRB has reviewed and approved if changes are required to the protocol during the study period.

7.4 Study duration

Each subject is expected to participate in the trial for approximately 14 to 15 months from the first visit to the last visit, and the study will last about 16 months in total.

8. Study group

8.1 Inclusion criteria

1) Volunteers between 18 and 59 years of age (all include) with full civil capacity；
2) The subject voluntarily agree to participate in the clinical trial, sign informed consent, and provide valid identification; understand and comply with trial protocol requirements;
3) Axillary temperature < 37.3℃;
4) Female subjects of childbearing age and male subjects agree to take effective contraceptive measures during the study.

8.2 Exclusion criteria for first dose inoculation

1) The population vital signs and physical examination results prescribed by the

protocol are clinically meaningful to the clinicians;

2) A history of severe allergies to any component of the test vaccine, including aluminum preparations, such as: anaphylactic shock, allergic laryngeal edema, allergic purpura, thrombocytopenic purpura, local allergic necrosis (Arthus reaction), dyspnea, angioneurotic edema, etc.; or any previous history of severe adverse reactions following vaccine or drug use.

3) A history of SARS and SARS-CoV-2 (Satisfy any of the following: ① A history of SARS and SARS-CoV-2-infection or disease history; ② During this SARS-CoV-2 epidemic, there was a history of contact with confirmed/suspected patients of the novel crown);

4) Having taken antipyretics or painkillers within 24 hours before the first dose of vaccination

5) Inoculate subunit vaccine and inactivated vaccine within 14 days before the first dose of vaccination, and inoculate live attenuated vaccine within 30 days;

6) Volunteers suffering from the following diseases:

① Patients with acute febrile illness;

② Suffering from digestive system diseases in the past 7 days (eg, diarrhea, abdominal pain, vomiting, etc.);

③ Suffering from congenital malformations or developmental disorders, genetic defects, severe malnutrition, etc.;

④ History of congenital or acquired immunodeficiency, autoimmune diseases, or treatment with immunomodulators within 6 months (such as hormones, monoclonal antibodies, thymosin, and interferon, etc.); However, topical medication (such as ointment, eye drops, inhalation or nasal spray) is permitted;

⑤ Volunteers who have been diagnosed with infectious diseases, such as: tuberculosis, viral hepatitis patients and/or human immunodeficiency virus HIV antibody positive or syphilis specific antibody positive

⑥ Having a neurological disease or neurodevelopmental dysplasia (such as migraine, epilepsy, stroke, seizure in the last three years, encephalopathy, focal neurological deficits, Guillain-Barre syndrome, encephalomyelitis or transverse myelitis); a

history or family history of mental illness;

⑦ functional asplenia and absence of or removal of the spleen for any reason;

⑧ Suffering from serious chronic diseases or the disease is in the advanced stage and cannot be controlled smoothly, such as diabetes, thyroid disease;

⑨ Severe liver and kidney disease; the respiratory diseases that currently require daily medication (such as chronic obstructive pulmonary disease [COPD], asthma) or any treatment for exacerbations of respiratory disease within the last 5 years (for example, asthma exacerbations); a history of severe cardiovascular disease (such as congestive heart failure, cardiomyopathy, ischemic heart disease, arrhythmia, conduction block, myocardial infarction, pulmonary heart disease) or myocarditis or pericarditis;

⑩ A history of thrombocytopenia, any coagulation dysfunction or anticoagulant treatment, or have obvious bleeding;

⑪ The cancer patients.

7) Having received blood or blood-related products, including immunoglobulin, within 3 months; or plan to use it during the research period

8) Women in lactation or pregnancy (positive urine pregnancy test test)

9) Using any research or unregistered products (drugs, vaccines, biological products or devices) other than research products within 3 months, or planning to use them during the research period;

10) The researchers believe that any disease or condition in the subject may put the subject at an unacceptable risk; the subject cannot meet the requirements of the protocol; the situation that interferes with the assessment of the vaccine response.

8.3 Exclusion criteria for persistent immune blood sampling

If subjects do not complete the full immunization, they will not receive humoral immunogenicity blood sampling at 6 months, 12 months after the full immunization and cellular immunogenicity blood sampling at 12 months after the full immunization (if applicable).

8.4 Suspension/termination of vaccination criteria

8.4.1 Suspension of vaccination contraindications

If any of the following symptoms occurs, the investigator will delay the vaccination until the situation resolves. The delay in vaccination should be as close to the specified time window as possible (refer to visit flow chart).

- Fever (axillary temperature ≥ 37.3 ℃ on the day of vaccination).
- Acute illness or in the acute onset of a chronic illness (within 3 days before vaccination).
- Inadequate intervals between doses of other vaccines (Any subunit inactivated vaccine shall not be administered within 14 days prior to inoculation, and any live attenuated vaccine shall not be administered within 30 days prior to inoculation).
- Other situations in which the researchers believe vaccination should be delayed.

If vaccination is delayed beyond the time interval allowed by the study, further discussion and determination of whether to remove the subject from the Per Protocol Set will be made jointly by the sponsor and principal investigator. However, the subject will continue to participate in the study and will be retained in the Full Analysis Set.

8.4.2 Criteria for termination of vaccination

The investigator will terminate the subject's vaccination with the testing vaccine if any of the following conditions occur.

1) A new discovery or a novel occurrence that meets the first exclusion criteria prior to vaccination (except for number 1, number 5 and 6 (i) and (ii)).

2) Severe reactions after vaccination (experimental vaccines or other vaccines).

3) High fever (axillary temperature > 39.5 ℃) within 48 hours after vaccination.

4) Other serious adverse events: the decision to discontinue the experimental vaccination is based on the therapeutic need for the vaccine.

5) Any other reason that the investigator assessed the need to discontinue the experimental vaccination.

8.5 Complete and withdraw from the study

8.5.1 Complete the study

Subjects who complete all visits as required by the protocol shall be deemed to have completed the study.

8.5.2 Withdraw from the study

A subject who withdraws from the study (shedding) is defined as: a subject unable to finish the last required visit according to trial protocol for any reason. After a subject withdraws from the study, the withdrawn subject will not be replaced. Withdrawal criteria are divided into investigator-determined withdrawal and subject-determined withdrawal.

Investigator-determined withdrawal:

1) An AE or concomitant condition occurs when the trial cannot be continued;

2) Subjects have participated in other clinical trials before the end of this clinical trial;

3) Other circumstances in which the investigator considers the subject unsuitable for continued participating in this clinical trail.

Subject-determined withdrawal: the subject has the right to withdraw from the study at any stage of the study. For example, the subject becomes intolerant of certain adverse effects and withdraws informed consent.

When a subject withdraws from the study, the investigator will fully inform the subject of the risks of withdrawal and subsequent precautionary treatment, and will distinguish subjects who withdraw due to adverse events from those who withdraw for other reasons. The investigator determines the relevant follow-up content based on the timing and circumstances of the subject's withdrawal.

The investigator should make every effort to contact subjects who fail to return for follow-up visit at the required time. After the subject withdraws from the study, the investigator shall provide the necessary guidance for the subject's emergence into trial-related clinical conditions and follow the AE/SAE prognosis.

Collect all data for analysis before the subject withdraws from the study/the date the subject was last contacted, the information related to the withdrawal from the study and the possible reasons that led to the withdrawal shall be recorded in eCRF , such as:

- Serious adverse events.
- Non-serious adverse events.
- Protocol violations.
- Pregnancy.
- Voluntary withdrawal (not as a result of an adverse event).

- Leaving the premises of the research center.
- Loss of visits.
- Death.
- Other (detailed description).

8.6 Handing of Lost visit

When a subject cannot return for visit on time, the investigator should make efforts to contact the subject on the premise of fully respecting the subject's rights, or at least determine the health status of the subject. And record the efforts made (for example, phone calls and SMS records).

8.7 Discontinuation of experimental vaccines

Subjects who have discontinued the trial vaccine are those who have not received the full vaccination. Subjects who discontinue experimental vaccines do not necessarily withdraw from the study. According to specific circumstances, the investigator can arrange for them to continue to complete other research procedures or visits (such as safety or immunogenicity) as required by the protocol.

8.8 Protocol deviation/violation

Protocol deviation: Refers to any changes and behaviors that do not follow the clinical trial protocol design or process, and are not approved by the ethics committee. Behaviors that do not affect the rights, safety and benefits of subjects, or the completeness, accuracy and reliability of test data, and the evaluation of safety or main indicators belong to a protocol deviation;

Protocol violation: Refers to any changes and behaviors that do not follow the clinical trial protocol design or process, and are not approved by the ethics committee, and this changes do affect the rights, safety and benefits of subjects, or affect the completeness, accuracy, reliability of test data, and the evaluation of safety or main indicators ,belong to a protocol violation.

- Protocol deviations include, but are not limited to:
  1) Visiting beyond the Window period.
- Protocol violations include, but are not limited to.
  1) Giving subjects the wrong group of vaccines or the wrong dose.
  2) Subjects using prophylactic medication (prophylactic use of antipyretics, analgesics, and allergy medications, receiving intramuscular, oral, or intravenously administered

systemic glucocorticosteroids or other immunosuppressive agents ⩾ 2 mg/kg/day for ⩾ 14 days during the recruitment period).

3) Vaccinated with unapproved experimental vaccines that have been subjected to cold chain disruption.

4) The subject met the withdrawal/termination criteria and was not withdrawn.

5) The investigator has not received IRB's approval for a change in protocol (except in an emergency to safeguard the interests of the subject).

6) Insufficient interval between vaccinations (inactivated or subunit vaccines received within 14 days before or after vaccination, or any live vaccine within 1 month, except for vaccination with an allowable vaccine after an experimental vaccine).

For the protocol deviation/violation during the research process, the on-site investigator should report the fact, process, cause and impact of the incident to the responsible agency. The principal investigator should give opinions on the handling of the incident, and the protocol violation should be reported to ethics committee.

Researchers should conduct targeted training on related staff involved in the protocol violation to prevent similar incidents from recurring, and record the training process.

8.9 Pregnancy events

Pregnancy is an exclusion condition for each dose of vaccination, and subjects are required to sign Informed Consent Form to take effective contraceptive measures within 12 months after the full course of immunization, but subjects may still get pregnant unexpectedly during the participation process. Pregnancy events that occurred after the subject was vaccinated to the entire study period should be reported, and the investigator should fill in the "Pregnancy Event Report Form".

Researchers closely follow pregnant subjects or subjects' partners to obtain information about pregnancy outcomes (for example, details of delivery and newborn conditions or termination of pregnancy), and update "the pregnancy incident report form". The condition of the newborn is followed up for 1 year, and the follow-up will be based on the non-clinical results and the 1-year observation results to decide whether to continue the follow-up

Pregnancy itself is not considered as SAE, but any complications during pregnancy will be considered as AE, and in some cases can be considered as SAE, such as: spontaneous abortion, stillborn foetus, stillbirth and infant congenital abnormalities. When no abnormality

is found in the fetus, the abortion due to the mother's personal decision is not considered an AE.

The handling of pregnancy events that occurred during vaccination is as follows:

- If the pregnancy occurs after the first dose of vaccination, but the full vaccination has not been completed, the female subject shall not participate in subsequent visits. The researcher will contact the subjects regularly to conduct pregnancy assessments until the end of pregnancy (neonatal follow-up for 1 year, miscarriage, abortion, etc.).
- If pregnancy is discovered after the subject has completed the full vaccination, the subject can complete the research visit according to the trial protocol and the investigator's decision.

8.10 Criteria for suspension/termination of trial

This trial or any other trail has obtained new data about the vaccine, the administrative department, sponsor, investigator, and/or IRB recommends suspension/termination of the trial; Suspended test criteria:

In any of the following situations, the trial needs to be suspended and immediately reported to the ethics committee, provincial and national drug administration departments; and DSMB experts will be urgently convened to conduct safety demonstration analysis to determine whether to continue the trial.

The event that caused the suspension of the trial	**Number of cases/ %**
Vaccine-related deaths or serious life-threatening adverse reactions occurred during the study period	⩾ 1 case
Severe or death cases after novel coronavirus infection occurred during the study period	⩾ 1 case
Grade 3 and above, lasting 48 h of adverse events occurred after any dose of vaccination	> 15% Number of vaccinated persons

Trial termination criteria:

In the following cases, the trial shall be terminated and immediately reported to the DSMB,

the ethics committee, provincial and national drug administration departments.

An event that results in the termination of a trial	**number of cases %**
Grade 3 and above, lasting 48 h of adverse events occurred after any dose of vaccination	>30% Number of vaccinated persons;

In the event of premature termination or suspension of the study, the sponsor will immediately notify the investigator, the ethics committee, and the drug regulatory authority of the reasons for the suspension or termination, as required by the appropriate registration regulations.

Regardless of the reason for premature termination of the study, the investigator shall immediately notify the subjects and ensure appropriate follow-up with the subjects.

9. Experimental vaccines and concomitant medication

9.1 Experimental vaccine

(1) Recombination Novel Coronavirus Vaccine (Low dose)

Production unit:	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.
Batch number:	See quality inspection report
Specification:	25 μg / 0.5 mL / bottle
Active ingredients:	Recombinant NCP-RBD protein 25 μg, aluminum hydroxide adjuvant 0.25 mg
Other:	See quality inspection report
Inspection entity:	National Institutes for Food and Drug Control
Inspection report number:	See quality inspection report
Validity period:	2 years (Tentative)

If the vaccine batch used in the trial is inconsistent with that recorded in the protocol, the responsible agency shall explain it to the ethics committee and record it (or in accordance with the requirements of the IRB) before the start of the clinical trial.

(2) Recombination Novel Coronavirus Vaccine (High dose)

Production unit:	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.
Batch number:	See quality inspection report
Specification:	50 μg / 0.5 mL / bottle
Active ingredients:	Recombinant NCP-RBD protein 50 μg, aluminum

	hydroxide adjuvant 0.25 mg
Other:	See quality inspection report
Inspection entity:	National Institutes for Food and Drug Control
Inspection report number:	See quality inspection report
Validity period:	2 years (Tentative)

If the vaccine batch used in the trial is inconsistent with that recorded in the protocol, the responsible agency shall explain it to the ethics committee and record it (or in accordance with the requirements of the IRB) before the start of the clinical trial.

(3) Recombination Novel Coronavirus vaccine placebo

Production unit:	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.
Batch number:	See quality inspection report
Specification:	0.5 mL / bottle
Active ingredients:	Aluminum hydroxide adjuvant 0.25mg, containing no antigen
Other:	See quality inspection report
Inspection entity:	the National Institutes for Food and Drug Control
Inspection report number:	See quality inspection report
Validity period:	2 years (Tentative)

9.2 Packaging and labelling

The outer packaging and inner packaging of the test vaccine are affixed with labels with the same number, and a self-adhesive label with the same number printed in advance is placed in the packaging box to paste the subjects' vaccination and follow-up records. The label contains the following information:

1)Vaccine bulk packaging label: product name, number range, storage conditions, company name, batch number, specification and expiration date. The bulk packaging box should be printed with words like "For clinical research only".

2)Single vaccine packaging label: product name, study number, vaccine specification, expiration date, batch number, dosage, initials of the subject, storage conditions, words like "For clinical research only", etc.

3)Vaccine vial label: vaccine number.

4)Built-in label (for sticking the vaccination and follow-up record book): vaccine number and subject's initials.

After the vaccine has been administered, the subject's initials must be written on the outer vaccine package label and the internal label (for sticking in the vaccination and follow-up record book), and the vaccinator must check that the vaccine is correct before administering the corresponding numbered vaccine.

9.3 Vaccine storage and transportation

The vaccine should be stored and transported in the dark at 2-8 ℃, and freezing is strictly prohibited. The storage temperature needs to be monitored and recorded daily (on the premise of maintaining automatic temperature monitoring and alarm, holidays can be arranged according to the specific situation on site). If the storage and transportation conditions exceed the specified range, the on-site researcher should immediately contact the clinical responsible institute’s personnel and the sponsor, and the experimental vaccine cannot be used until the sponsor's opinion is obtained.

9.4 Vaccine usage

Shake well before use and visually observe whether there are particles. If there are clots and foreign bodies that cannot be shaken, do not use.

- Vaccination site and route: intramuscular injection into the deltoid muscle of the upper arm
- Inoculation dose: 0.5 mL/ bottle
- Vaccination procedure: Subjects in the 2-dose groups are given 1 dose of experimental vaccine each time in 0, 1 month, and a total of 2 doses are given throughout the whole course. Subjects in the 3-dose group are given 1 dose of experimental vaccine each time at 0,1,2 months, and a total of 3 doses are given throughout the course.

9.5 Remind and prevent

As with all vaccines, the vaccination site should have appropriate emergency treatment measures and be equipped with epinephrine and other medicines for use in case of occasional severe allergic reactions after vaccination. Subjects should be observed on-site for 30 minutes after vaccination.

After vaccination (at least 1 dose of test vaccine) (at each visit), remind the subject to be aware of fever and/or respiratory symptoms (such as dyspnea, sore throat, etc.) and suspected or confirmed COVID-19 cases during the study period and contact the investigator in time. If

the subject is suspected of being infected with SARS-CoV-2 or confirmed to be infected with SARS-CoV-2 during the trial, he must go to a local designated hospital for hospitalization for diagnosis and treatment.

9.6 Concomitant medication

After vaccination and at each visit/contact of the diary card/contact card, the researcher should ask the subject whether he has taken any medications and vaccinated. All concomitant medications/vaccines (except vitamins and/or food supplements) should be recorded in the diary card/contact card.

Concomitant medication: Refers to the vaccine subjects used from the time signed informed consent to the vaccination within 30 days after the last dose of the vaccination in addition to the experimental vaccine of all drugs and the last one dose of vaccine for treatment of SAE 30 days to 12 months or used drugs in the treatment of pregnancy complications, including antibiotics, antipyretic analgesics, allergy medicines, biological products (vaccine), medium (into) drugs (except vitamin and/or food additives)

The data management personnel should classify the combined medications according to the following 10 categories:

(1) Hormone/steroid drugs and other immunosuppressants;

(2) Anti-allergic drugs;

(3) Antipyretic/analgesic/non-steroidal anti-inflammatory drugs;

(4) Vaccines and biological products;

(5) Immunoglobulin and other blood products;

(6) Antibiotics;

(7) Antiviral drugs;

(8) Chinese patent medicine;

(9) Traditional Chinese medicine prescription;

(10) Others.

Permissible vaccines: Follow the inclusion/exclusion criteria for vaccine use, and there should be no restrictions on the use of vaccines for emergencies such as rabies or tetanus, but the use of vaccines should be documented as required. Other vaccines such as subunit and inactivated vaccines administered prior to administration of the trial vaccine should be administered at least 14 days apart from the trial vaccine, and live attenuated vaccines should be administered at least 30 days apart from the trial vaccine.

Permissive medication: Subjects who experience an adverse event during the trial shall be permitted to receive the necessary medication, and medication information shall be recorded

truthfully as required. Contraceptive use should also be allowed in this trial because of the contraceptive requirements for the subject; however, any medication used should be documented as required.

Prophylactic medication: A medication given when there are no symptoms and expected vaccination reactions. If the subject uses aspirin to treat heart disease, it is a "reportable" combination drug, but not a "preventive drug"; if the subject does not have a fever during the enrollment period, take antipyretic drugs to prevent fever. At times, antipyretic drugs are regarded as preventive drugs. When enrolling in the group, you should ask about the drugs being used to confirm that the subjects have not used antipyretic, analgesic and anti-allergic drugs

The following drugs/preparations should be collected throughout the study period. The use of the following drugs may affect the subject's entry into the PPS (Per Protocol Set) :

• Use any research or unregistered products (drugs, vaccines) other than experimental vaccines during the research period;

• Long-term use (≥14 days) immunosuppressant or other immunomodulatory drugs (such as glucocorticoid drugs);

• Inject immunoglobulin and other immune boosters after the first dose.

During data cleaning, the data administrator will establish a detailed and comprehensive list of reasons to exclude from the basic immune PPS analysis set, and make a decision on whether to include the PPS analysis when reviewing the decision.

9.7 Subjects compliance

During the trial, the compliance of subjects was evaluated, including:

- Complete the entire vaccination process in accordance with the immunization procedures within the allowed time interval;
- After vaccination, within the allowed time interval, follow-up sampling compliance.

According to the subject’s degree of compliance and considering its impact on the effectiveness evaluation, if the sponsor and the main investigator discuss and determine that the subject’s compliance is poor, the data will be excluded from the analysis of the compliance plan.

10. Trial procedure

10.1 Volunteer recruitment

After the trial site is determined and approved by the Ethics Committee, before the start

of the study, the investigator or its authorized person will issue a recruitment notice to volunteers who meet the requirements of entry age, vaccination history, and health status, and contact, register and invite them to participate in this research.

10.2 Informed consent

The informed consent process should be completed before any research procedures. Before joining the group, the investigator informed the volunteers of the relevant information about the clinical trial, and the volunteer and the investigator signed a written informed consent form in duplicate. The volunteers kept a copy, and the original was kept at the study site. After signing the informed consent, the researcher needs to train the subjects on epidemic safety protection.

10.3 Screening

Researchers conducted inquiries based on the "inclusion and exclusion criteria" to obtain volunteers' medical history, travel history, occupation and other information, and performed relevant examinations on all volunteers, including height, weight, vital signs, physical examination (skin, heart and lung auscultation) , women of child bearing age need a urine pregnancy test test. A medical history inquiry and vital signs measurement were performed on the day of vaccination. Researchers need to fill in screening information and demographic data (such as date of birth, gender, and race) in the vaccination and follow-up record brochure.

10.4 Assigning study number

Subjects qualified for screening will be given study numbers in order. The study number is used to identify all procedures that occurred after the subjects were randomized. Once the study number is assigned to a subject, it cannot be reassigned to other subjects; the number of the dropped subjects will not be reassigned regardless of whether they are vaccinated or not. The researcher fills in the subject's screening number and initials in the random allocation form, and fills in the vaccine number (same study number) in the vaccination and follow-up record brochure.

10.5 Sample collection before immunization

10.5.1 Sampling blood of immunogenicity

Humoral immunity: 8 mL blood samples were collected from all subjects before the first dose of vaccination for detection of neutralizing antibody, S protein binding antibody (IgG), RBD protein binding antibody (IgG)

Cellular immunity: There are 72 subjects in each immunization program group, a total of 144 subjects (24 cases of low dose in 2-dose group, 24 cases of high dose in 2-dose group, 24 cases of placebo in 2-dose group; 24 cases of low dose in 3-dose group, 24 cases of high dose

in 3-dose group, 24 cases of placebo in 3-dose group). Blood samples (30 ml) will be collected before the first dose of vaccination, for detection of cytokine IL-2, IL-4, IL-5, IL-6 and IFN-γ. The plasma produced in the process of lymphocyte collection and separation is used for research on alternative methods of neutralizing antibodies.

10.5.2 Urine collection

For fertile woman, 5.0-10.0 mL of urine should be collected for urine pregnancy test testing during the screening period, before the second dose of vaccination, and before the third dose of vaccination (if applicable).

10.5.3 Other samples

Pharyngeal swabs were collected for SARS-COV-2 real-time fluorescent RT-PCR nucleic acid detection for all subjects after assigned study number, and about 4.0 mL blood was collected for SARS-CoV-2 IgM and IgG antibody detection.

10.6 Vaccination

Obtain the corresponding numbered vaccine according to the assigned study number. After checking the number on the vaccination and follow-up record brochure, fill in the subject's initials on the vaccine packaging label, fill in the vaccine's built-in label and paste it on the designated location of the vaccination and follow-up record book. Check subject's information again before vaccination. Shake the vaccine well and inject 0.5mL vaccine into the deltoid muscle of the upper arm.

10.6.1 First dose of vaccination

According to the random allocation number, the subjects completed the first dose of vaccination on the day of enrollment (D0).

10.6.2 Subsequent doses of vaccination

Subjects in the 2-dose group receive the second dose of vaccination on the 30th day after the first dose of vaccination, and the vaccination window period is +7 days. In the 3-dose group, the second dose is inoculated on the 30th day after the first dose of vaccination, and the third dose is inoculated on the 30th day after the second dose. The inoculation window period is +7 days. If the subject meets the criteria for suspension/termination of vaccination (refer to section 7.3), please postpone or stop the trial vaccination.

10.7 Medical observation

After each dose of test vaccine, the subject should stay on site for 30 minutes. The researcher will issue a thermometer (issued on the day of the first dose of vaccination), measuring ruler (issued on the day of the first dose of vaccination), and diary cards to the subject, and instruct them to measure and record their body temperature (axillary temperature)

within 7 days after vaccination), adverse events and fill in the diary card, make an appointment to retrieve the diary card and issue the contact card.

The investigator emphasized to the subjects the investigator phone number on the copy of the informed consent form/diary card/contact card, etc., and instructed them to contact investigator if they experience any signs or symptoms (especially fever and/ or respiratory symptoms) or COVID-19 Suspected or confirmed cases or an event requiring hospitalization occurs. If the subject is suspected or confirmed to be infected with SARS-CoV-2 during the trial period, he must go to a designated local hospital for diagnosis and treatment.

10.8 Sample collection after immunization

Humoral immunity:

Before the second dose of vaccination (window period + 7 days), 14 days after each dose of vaccination (window period ± 1 day), 1 month after the entire vaccination period (window period + 7 days), 6 months (window period + 30 Days) and 12 months (window period + 30 days), subjects in the 2-dose group are collected about 8 mL of venous blood for post-immunization SARS-CoV-2 neutralizing antibody, S protein binding antibody (IgG), RBD protein Binding antibody (IgG) detection.

Before the second dose of vaccination (window period + 7 days), before the third dose of vaccination (window period + 7 days), 14 days after each dose (window period ± 1 day), 1 month after the entire vaccination period (window period + 7 days), 6 months (window period + 30 days) and 12 months (window period + 30 days), subjects in the 3-dose group are collected about 8 mL of venous blood for post-immunization SARS-CoV-2 neutralizing antibody, S protein binding antibody (IgG), RBD protein Binding antibody (IgG) detection.

Cellular immunity:

About 30 ml of venous blood is collected from 144 subjects (72 subjects in different immune procedure groups, 24 cases of low dose in 2-dose group, 24 cases of high dose in 2-dose group, 24 cases of placebo in 2-dose group; 24 cases of low dose in 3-dose group, 24 cases of high dose in 3-dose group, 24 cases of placebo in 3-dose group), at 4 days (window period + 3 days) and 12 months (window period + 30 days) after whole-course immunization for post-immunization cytokine levels of IL-2, IL-4, IL-5, IL-6 and IFN-γ detection.

10.9 Safety follow-up and evaluation

10.9.1 Follow-up time and method

- ✓ Observation of immediate adverse events on site for 30 minutes after each dose of vaccination.
- ✓ A diary card with information on adverse events is issued to the subject after each dose of vaccination, and the subject's temperature is taken and adverse events recorded daily for 0-7 days after vaccination, as required by the diary card.
- ✓ Diary cards were collected on day 8 after each dose of vaccine, and the investigator reviewed and recorded adverse events, while subjects were issued contact cards for recording adverse events for 8-30 days after each dose of vaccine.
- ✓ Collection of all serious adverse events and pregnancy events by telephone/information after the first dose of vaccination and through the end of the study, with the last dose of contact card retrieved to alert subjects to contact the investigator promptly in the event of any signs or symptoms (especially fever and/or respiratory symptoms), the occurrence of suspected or confirmed cases of COVID-19, SAE and pregnancy events during the study that they consider serious.
- ✓ At each visit the researcher emphasized that subjects could contact the researcher at any time via the phone number on the diary card/contact card/copy of the informed consent form etc.

10.9.2 Content of the follow-up

The safety observation includes all AEs (including collective and non-solicited AEs) from the first dose of the subject to 30 days after the full course of vaccination, and all SAEs and pregnancy events from the first dose to 12 months after the full course of vaccination.

10.9.2.1 Collective adverse events

Solicited AE: The following events occurred within 7 days after vaccination.

Inoculation site (local) adverse reactions	Pain, swelling, induration, flushing, rash, itching
Vital signs	Fever

Non-inoculation site (systemic) adverse reactions	Headache, fatigue, nausea, vomiting, diarrhea, muscle pain, cough, acute allergic reaction, mental disorder (specific symptoms)

10.9.2.2 Non-Collective adverse events

Non-collective adverse events includes all other AEs reported other than solicited AEs from the first dose of vaccination to 30 days after full inoculation, including the collection of the outside of the specified collection time window collective adverse events reported (for example: if the solicited AE occurs after 8 days of vaccination, it can be a non-solicited AE record).

10.9.2.3 Serious adverse event

A serious adverse event (SAE) is a medical event that:：

1) Death-causing;
2) Life-threatening;
3) Permanent or severe disability or loss of function;
4) Hospitalization or prolonged hospitalization;
5) Congenital anomalies or birth defects, etc.

10.9.3 Safety assessment

10.9.3.1 Severity of adverse events

Observe and determine the severity of adverse events in accordance with the *Guidelines for the Classification of Adverse Events in Preventive Vaccine Clinical Trials* (referred to as the *Guidelines for Classification Standards*) issued by NMPA on December 31, 2019. For uninvolved adverse events, the intensity is evaluated according to the *other adverse event classification* standard. According to the characteristics of the subjects in this trial, the indicators have been adjusted accordingly. The severity of adverse events is classified as follows:

Inoculation site (local) adverse event classification table

Symptoms/ signs	Level 1	Level 2	Level 3	Level 4
Pain	Does not affect or slightly affects the physical activity	Affect physical activity	Affect daily life	Loss of basic self-care ability, or hospitalization
Induration*	2.5 ~ <5cm in diameter or 6.25 ~ <25cm^2 in area with no or slight impact on daily	5～<10 cm in diameter or 25 ~ <100 cm^2 in area with no or impact on daily life	With diameter ≥10cm or area ≥100cm^2 or ulceration or secondary infection or phlebitis or sterile abscess or wound	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis

	life		drainage or seriously affecting daily life	
Blush#**	2.5 ~ <5cm in diameter or 6.25 ~ <25cm^2 in area with no or slight impact on daily life	5～<10 cm in diameter or 25 ~ <100 cm^2 in area with no or impact on daily life	With diameter ⩾10cm or area ⩾100cm^2 or ulceration or secondary infection or phlebitis or sterile abscess or wound drainage or seriously affecting daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Swelling#**	2.5 ~ <5cm in diameter or 6.25 ~ <25cm^2 in area with no or slight impact on daily life	5～<10 cm in diameter or 25 ~ <100 cm^2 in area with no or impact on daily life	With diameter ⩾10cm or area ⩾100cm^2 or ulceration or secondary infection or phlebitis or sterile abscess or wound drainage or seriously affecting daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Pruritus	Pruritus at the inoculation site is relieved on its own or within 48 h after treatment	Pruritus at the inoculation site is not relieved within 48 h after treatment	impact on daily life	NA
Eythra*	2.5 ~ <5cm in diameter or 6.25 ~ <25cm^2 in area with no or slight impact on daily life	5～<10 cm in diameter or 25 ~ <100 cm^2 in area with no or impact on daily life	With diameter ⩾10cm or area ⩾100cm^2 or ulceration or secondary infection or phlebitis or sterile abscess or wound drainage or seriously affecting daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis

****In addition to the direct measurement of diameter for grading evaluation, the progress of measurement results should also be recorded**

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

#The evaluation and grading of induration, blush, swelling and rash should be based on functional grade and actual measurement results, and higher ranking indicators should be selected.

Vital signs/Non-inoculation site (systemic) adverse event classification table

Symptoms/ signs	Level 1	Level 2	Level 3	Level 4
Fever [axillary temperature (℃)]	37.3～<38.0	38.0～<38.5	38.5～<39.5	⩾39.5, lasts for more than 3 days
Headache	Does not affect daily activities and does not require treatment	The pain is transient, slightly affecting daily activities and may require treatment or intervention	Severely affecting daily activities and requiring treatment or intervention	Refractory cases require emergency treatment or hospitalization
Fatigue/weakness	does not affect daily activities	Affect normal daily activities	Seriously affecting daily activities, unable to work	Emergency or hospitalization

Nausea	Transient nausea (<24 hours) or intermittent and food intake is basically normal	Persistent nausea leads to reduced food intake (24 to 48 hours)	Persistent nausea results in almost no food intake (>48 hours) or the need for intravenous fluids	Life threatening (e.g. hypotensive shock)
Vomiting	1~2 times /24 hours without affecting the activity	3 ~ 5 times /24 hours or limited activity	>6 times within 24 hours or intravenous fluids may be required	Shock requiring hospitalization or other means of nutrition due to hypotension
Diarrhea	Mild or transient, 3-4 times per day, abnormal fecal characteristics, or mild diarrhea lasting less than 1 week	Moderate or persistent, 5 ~ 7 times per day, abnormal fecal characteristics, or diarrhea > for 1 week	>7 times/day, abnormal stool properties, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, intravenous infusion is required >2L	Hypotensive shock requiring hospitalization
Muscle pain	does not affect daily activities	Slightly affecting daily activities	Severe muscle pain, which severely affects daily activities	Emergency or hospitalization
Cough	Transient cough requires no treatment	Persistent cough is effective	The coughing fits are out of control	Emergency or hospitalization
Acute allergic reaction**	Local urticaria (blister) does not require treatment	Local urticaria requiring treatment or mild angioedema without treatment	Extensive urticaria or angioedema requires treatment or mild bronchospasm	Anaphylactic shock or life-threatening bronchospasm or laryngeal edema
Mental disorders (including anxiety, depression, mania, and psychosis) should be reported in detail	Mild symptoms, no need to see a doctor or behavior does not affect or slightly affect daily life	Have clinical symptoms, need to see a doctor or behavior affects daily life	Need to be hospitalized or unable to support daily life	Have a tendency to harm oneself or others, or acute mental confusion or loss of basic self-care ability
Other adverse event rating#(for uninvolved adverse events, the intensity is assessed according to this standard)	Mild: short-term (< 48h) or mild discomfort, does not affect activities, no treatment	Moderate: Mild or moderate activity limitation, may require medical treatment, no or only mild treatment	Severe: obviously restricted activities, need to see a doctor and receive treatment, may need to be hospitalized	Critical: may be life-threatening, severely restricted activities, need monitoring and treatment

****refers to type I hypersensitivity.**

[#]The classification of other adverse events is the general principle in the guiding principles of the classification standard, and the severity of "death" is "level 5"

10.9.3.2 Correlation with experimental vaccines

For the expected or unexpected AE (collective or non- solicited AE) happened in this trial, the researchers should determine its relevance to vaccination in time, and found SAE associated with vaccination in the process of clinical trials and the groups and tendency of adverse events, suspend or terminate clinical trials in time to reduce harm to the participants at the maximum extent.

General principles of relevance judgment:

- ✓ **Certainly related:** there is evidence of trial vaccination; the time sequence of the occurrence of adverse events and trial vaccination is reasonable; the occurrence of adverse events is explained by the trial vaccine more reasonable than other reasons; the repeated vaccination of the trial vaccine is positive; the adverse event situation is consistent with the prior knowledge of this or this type of vaccine.
- ✓ **It is likely to be related:** there is evidence of test vaccine; the time sequence of the occurrence of adverse events and vaccination is reasonable; the occurrence of adverse events is explained by the vaccination more reasonable than other reasons.
- ✓ **It may be related:** there is evidence of test vaccine; the time sequence of the occurrence of adverse events and vaccination is reasonable; the occurrence of adverse events cannot be ruled out caused by the test vaccination, but may also be caused by other reasons.
- ✓ **Possibly irrelevant:** there is evidence of test vaccine; adverse events are more likely to be caused by other reasons; repeated vaccination trials are negative or uncertain.
- ✓ **Certainly irrelevant:** the subject did not use the trial vaccine; or the occurrence of adverse events and the time sequence of the trial vaccinations were not logical; or there were other significant reasons that could lead to the adverse events.

In statistical analysis, “definitely related”, “probably related” and “may be related” are all analyzed as “related” AEs related to the test vaccine; it is should be analyzed that “may be unrelated” and “definitely not related” are all considered “unrelated” to the test vaccine AE.

Because the researcher’s understanding of AE/SAE is a gradual process, the initial report may provide inaccurate correlation information. In subsequent visits, as the information is continuously added or updated, the researcher may change his initial perception of the correlation. Judgment of AE/SAE should be tracked at this time, especially SAE follow-up needs to update corresponding information.

10.9.3.3 Expectation

The investigator and sponsor should determine whether serious adverse events associated with the experimental vaccine are expected or unexpected. An adverse event shall be considered unexpected if its nature, severity or frequency is inconsistent with the previously described risk information for the research intervention.

10.9.3.4 Evaluation of prognosis

AE regression endings include.

Recovery: AE with or without treatment and subsequent complete disappearance of symptoms with no residual symptoms.

Remission: AEs improve with treatment.

Disappearance of symptoms but sequelae: AEs are treated and symptoms have disappeared but sequelae remain (the name or manifestation of the sequelae should be noted in the record).

Not cured/not in remission: AEs do not improve/do not recover from symptoms after treatment.

Death: leads to the end of life (cause and time of death must be collected).

Unknown: Unknown condition, failure to follow up and document, or subject refusal.

10.10 Immunogenicity assessment

Humoral immunity: Blood samples will be collected before each dose of vaccination, 14 days after each dose of vaccination, 1 month, 6 months and 12 months after the entire inoculation, and tested for SARS-CoV-2 neutralizing antibody, S protein binding antibody (IgG) and RBD protein binding antibody (IgG).

Cell-mediated immunity: Blood samples will be collected from subjects in different immune procedure groups (24 cases of low dose in 2-dose group, 24 cases of high dose in 2-dose group, 24 cases of placebo in 2-dose group; 24 cases of low dose in 3-dose group, 24 cases of high dose in 3-dose group, 24 cases of placebo in 3-dose group) before the first dose of vaccination, on the 4th day and 12 months after the full course of immunization, for cytokine IL-2, IL-4, IL-5, IL-6 and IFN-γ detection.

10.11 Biological sample processing and testing

10.11.1 Urine sample for pregnancy test

For fertile woman, a urine pregnancy test test is required. The precautions for collecting urine are detailed in the SOP related to the urine pregnancy test test.

10.11.2 Immunogenic blood sample processing

Humoral immunity: The volume of blood collection is about 8 mL, and hemolysis should be avoided during collection. The centrifuged serum is divided into 6 tubes (serum cryopreservation tubes) in a sterile environment, each tube is about 0.5 mL, 5 of them are sent for testing, and the last one is kept as a backup.

Cellular immunity: Use anticoagulant vacuum blood collection tube to collect about 30mL of venous blood. During/after collection, it is necessary to gently reverse it several times in time and mix it evenly to avoid blood clots. The separated lymphocytes are used for the detection of cellular immunological indicators, and at the same time, the plasma (about 3 mL) produced during the separation process is divided into 3 tubes, each tube is about 1mL, 2 tubes are sent for inspection and 1 tube is saved as a backup. Research on alternative methods of detection methods used to replace pseudovirus/live virus neutralizing antibodies (competitive ELASA Neutralizing Antibody Detection Method) and other immunological methodology research.

For the specific steps and methods of blood sampling for humoral immune and cellular immunity, such as centrifugation, aliquoting, freezing and transportation, see the SOP.

10.11.3 Biological sample numbering rules

The blood sample number used for trial is “ID-N”, where ID represents the subjects’ study number, N is the serial number of blood sampling, such as:

· The number of the first blood collection (blood collection before exemption) is: ID-1;

· The second blood sampling number is: ID-2, and so on.

10.11.4 Biological sample detection

Detection quality control standard is provided by the National Institutes for Food and Drug Control, in view of novel coronavirus RBD antibody and neutralize antibody have not already approved the detection reagent that appear on the market or general method, detect qualitative control article by vaccine development party and the National Institutes for Food

and Drug Control negotiate solve. Novel Coronavirus (SARS-COV-2) neutralizing antibody and IgG titer are tested by the National Institutes for Food and Drug Control.

11. Data management

11.1 The design of eCRF

eCRF is designed according to the test procedures and flow charts specified in the protocol. After the first draft is formed, it needs to be reviewed by project managers, data and statisticians, plan writers and other project team members. The eCRF complies with the plan and complies with relevant laws and regulations, and the version control process needs to be fully recorded.

11.2 eCRF filling guide

The eCRF filling guide is based on the specific filling instructions for each page of eCRF and each data point according to the research plan. Ensure that the clinical trial center obtains the eCRF and its filling guidelines before the subjects are selected. Train the relevant staff of the clinical trial center on the plan, eCRF filling and data submission process, and the process needs to be archived and recorded.

11.3 eCRF notes

eCRF notes is the annotation of blank eCRF, recording the location of each eCRF data item and the variable name and encoding in the database, and it needs to be reviewed by DM.

11.4 Database design

The database should be established in accordance with the data set name, variable name, variable type and variable length in the eCRF notes, and try to follow the standard database structure and settings. After the establishment of the database is completed, a database test should be carried out and a database test report should be issued, which should be signed and confirmed by the manager in charge of data management.

11.5 Permission assignment

System administrators create accounts and grant different permissions according to different roles.

11.6 eCRF filling

Researchers need to collect subjects' data in accordance with the requirements of the trial protocol, and fill in the eCRF accurately, timely, completely, and standardly according to the original information and the filling guide. The modification of eCRF data must comply with standard operating procedures and retain traces of modification.

11.7 Questioning and resolution

The data management department DM lists a detailed data verification plan. The verification plan is reviewed by the sponsor, medical staff, statistician, project manager, etc., and then signed and confirmed by the data administrator, data manager, and sponsor. After the data is entered into EDC, the system will verify the data according to the Edit Check established in the data verification plan, and questionable data will be automatically questioned. The data that cannot be set as the system to issue a question will be sent to the manual question through EDC. The input personnel or researcher will confirm and answer the manual question and the system question, and modify the wrong data if necessary until the question is resolved. When the question is not resolved, the data administrator and clinical monitor can question the data point again, and all traces are stored in the EDC database.

11.8 Data modification and review

Data entry personnel or researchers can modify the data after verifying the data. The modified data should be prompted by the system and the reason for the modification should be filled in the system. The researcher has the authority to review all final data.

11.9 Medical coding

Adverse events collected in clinical trials are coded using a standard dictionary. The standard dictionary generally used is MedDRA. The encoded data set should clearly record the dictionary and version used during encoding.

11.10 SAE consistency comparison

Use the program to compare all SAE-related data points in the database with the data points in the PV (Pharmacovigilance) system. Inconsistent data needs to be communicated with PV personnel until there is no difference in the data.

11.11 Data review meeting

Before the database is locked, prepare the first draft of the data review report and all the data lists, and the sponsor, researcher, data manager and statistician will jointly finalize the database review. And conduct statistical analysis of population division, check serious adverse event reports and treatment records, check ADE/VED reports and treatment records, etc., according to the clinical trial protocol. After the data review meeting, the data review report and the population division plan should be finalized.

11.12 Database locking and unlocking

Database locking is an important milestone in the clinical research process. The locking

process and time should be clearly documented. Locking is to cancel the right to edit the database. Any unauthorized account cannot operate the database.

After the database is locked, if there is any modification, an application must be submitted, and it can be executed after the sponsor, investigator, statistician, clinical monitor, and data management personnel discuss and sign for confirmation, and record the reasons for unlocking in detail.

12. Statistical considerations

12.1 Research hypothesis

Not applicable.

12.2 Study endpoint

Refer to the chapter "6. Study End points" for the definition of study end points.

12.3 Considering the sample size

A total of 900 subjects will be enrolled in the trial, including 150 cases in the low-dose group with 2 doses, 150 cases in the high-dose group with 2 doses, 150 cases in the placebo control group with 2 doses, 150 cases in the low-dose group with 3 doses, and 150 cases in the high dose group, and 150 cases in the placebo control group with 3 doses. It is assumed that the positive rate of neutralizing antibody reached 80% in the vaccine group and 30% in the placebo group, and the test level was unilateral α =0.025. With this sample size, the difference between vaccine and placebo can be found with 99.99% confidence.

12.4 Analysis set

Full Analysis Set (FAS): Include all subjects who follow the principles of intention analysis (ITT), have been randomized, received at least one dose of vaccination; completed pre-immune blood sampling, and had valid antibody values. Among them, subjects who received the wrong vaccine are randomly assigned to receive immunogenicity evaluation according to ITT principle.

Per Protocol Set 1, (PPS1): Include all subjects who have not violated the inclusion/exclusion criteria, have been randomized, and have completed the first dose of immunization, completed the sampling of pre-immunization and 14 days after the first dose of immunogenicity evaluation, and have a valid antibody value. Among them, those meet the following conditions cannot enter PPS1:

- Those who violate the trial protocol and may affect the analysis results before immunogenic blood sampling 14 days after the first dose of vaccination;
- Those who received the wrong number of first dose vaccine;

- Those who use vaccines or drugs prohibited by the protocol 14 days after the first dose of immunization before immunogenicity blood collection;
- Other conditions affecting the evaluation of immunogenicity 14 days after the first dose of immunization.

Per Protocol Set 2, (PPS2): Include all subjects who have not violated the inclusion/exclusion criteria, have been randomized, and have completed the first dose of immunization, completed the pre- immunization and the second dose of immunogenicity evaluation, and have effective antibody values. Among them, those meet the following conditions cannot enter PPS2:

- Those who violate the trial protocol before the second dose of immunogenic blood sampling and may affect the analysis results;
- Those who received the wrong number of first dose vaccine;
- Those who use vaccines or drugs prohibited by the protocol before the second dose of immunization before immunogenicity blood collection;
- Other conditions affecting the evaluation of immunogenicity before the second dose of immunization.

Per Protocol Set 3, (PPS3): Include all subjects who have not violated the inclusion/exclusion criteria, have been randomized, and have completed the first 2 doses of immunization, before completing the immunization and 14 days after the second dose of immunogenicity evaluation, and have a valid antibody value. Among them, those meet the following conditions cannot enter PPS3:

- Those who violate the trial protocol and may affect the analysis results before immunogenic blood sampling 14 days after the second dose of vaccination;
- Those with the wrong vaccine number in the first two doses;
- Those who use vaccines or drugs prohibited by the protocol 14 days after the second dose of immunization before immunogenicity blood collection;
- Other conditions affecting the evaluation of immunogenicity 14 days after the second dose of immunization.

Per Protocol Set 4, (PPS4): Include all subjects who have not violated the inclusion/exclusion criteria, are randomly divided into 3 doses, completed the first two doses of immunization, completed the pre-immunity and the third dose of immunogenicity evaluation, and have effective antibody values. Among them, subjects who meet the following conditions are not allowed to enter PPS4

- Those who violate the trial protocol before the third dose of immunogenic blood

sampling and may affect the analysis results;

- Those with the wrong vaccine number in the first two doses;
- Those who use Vaccines or drugs prohibited by the protocol before the third dose of immunization before immunogenicity blood collection;
- Other conditions affecting the evaluation of immunogenicity before the third dose of immunization.

Per Protocol Set 5, (PPS5): Include all subjects who have not violated the inclusion/exclusion criteria, have been randomized and grouped, and completed the full vaccination according to the immunization program specified in the plan, and the blood samples are collected for cellular immunogenicity evaluation before and 4 days after the full vaccination and have effective antibody values. Among them, subjects who meet the following conditions are not allowed to enter PPS5:

- Those who violate the trial protocol and may affect the analysis results before cellular immunogenic blood sampling 4 days after the full immunization;
- Those with the wrong vaccine number;
- Those who use Vaccines or drugs prohibited by the protocol 4 days after the full immunization before cellular immunogenic blood collection;
- Other conditions affecting the evaluation of cellular immunogenicity 4 days after the full immunization.

Per Protocol Set 6, (PPS6): Include all subjects who have not violated the inclusion/exclusion criteria, have been randomized and grouped, and completed the full vaccination according to the immunization program specified in the plan, and the blood samples are collected for cellular immunogenicity evaluation before and 14 days after the third dose of immunization and have effective antibody values. Among them, subjects who meet the following conditions are not allowed to enter PPS6:

- Those who violate the trial protocol and may affect the analysis results before cellular immunogenic blood sampling 14 days after the third dose of immunization;
- Those with the wrong vaccine number;
- Those who use Vaccines or drugs prohibited by the protocol 14 days after the third dose of immunization before cellular immunogenic blood collection;
- Other conditions affecting the evaluation of cellular immunogenicity 14 days after the third dose of immunization.

Per Protocol Set 7, (PPS7): Include all subjects who have not violated the selection/ exclusion criteria, have been randomized into groups, and completed the full vaccination

according to the immunization program specified in the plan, completed the immunogenicity evaluation before and 30 days after the full vaccination, and have effective antibody values. Among them, subjects who meet the following conditions are not allowed to enter PPS7:

- Those who violate the trial protocol and may affect the analysis results before cellular immunogenic blood sampling 30 days after the full immunization;
- Those with the wrong vaccine number;
- Those who use Vaccines or drugs prohibited by the protocol 30 days after the full immunization before cellular immunogenic blood collection;
- Other conditions affecting the evaluation of cellular immunogenicity 30 days after the full immunization.

FAS and PPS1 are used for immunogenicity evaluation 14 days after the first dose of vaccination; FAS and PPS2 are used for the immunogenicity evaluation before the second dose of vaccination; FAS and PPS3 are used for the immunogenicity evaluation 14 days after the second dose of vaccination; FAS and PPS4 are used for the immunogenicity evaluation before the third dose of the 3-dose procedure; FAS and PPS5 are used for the evaluation of cellular immunogenicity 4 days after the whole course of immunization; FAS and PPS6 are used for immunogenicity evaluation 14 days after the whole course of immunization; FAS and PPS7 are used for immunogenicity evaluation 1 month after the whole course of immunization.

Safety Set, (SS): Include all subjects who have received at least one dose of vaccine. Among them, the subjects who receive the wrong vaccine number were evaluated according to the principle of ASaT (All Subjects as Treated) according to the vaccine group they actually received.

The safety analysis of each dose was carried out according to the actual number of inoculated persons per dose. The first dose of safety set includes all subjects who received the first dose of vaccine, denoted as SS1; the second dose of safety set includes all subjects who received the second dose of vaccine, denoted as SS2; the third dose of the safety set includes all subjects who received the third dose of vaccine, denoted as SS3.

Immune Persistence Set 6, (IPS6)

Include all subjects who have entered the immune persistence evaluation, collected blood for the immune persistence evaluation 6 months after completing the full course of immunity,

and have effective antibody values. PS6 is mainly used for the evaluation of immunity persistence 6 months after the whole course of immunity

Immune Persistence Set 12, (IPS12)

Including all subjects who have entered the immune persistence evaluation, 12 months after the completion of the whole course of immune persistence evaluation, and have effective results. The IPS12 of 12 months after the full vaccination is mainly used for the evaluation of the immunity persistence 12 months after the full vaccination.

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

12.5 Statistical analysis methods

12.5.1 General principle

The measurement data are statistically described by the mean, median, standard deviation, maximum and minimum; count data or grade data are expressed by frequency and relative frequency.

All statistical analysis is done using statistical software SAS 9.4.

12.5.2 Trial enrollment and completion

Summarize the number of subjects in the screening, randomization and completion of the basic immunization phase, completion of the 6-month and 12-month immune persistence phase, and the number of subjects in each analysis set, and analyze the reasons for the falling subjects. List the list of failed subjects, the list of dropped subjects, and the list of subjects who did not enter each analysis set.

12.5.3 Immunogenicity analysis

Calculate the positive conversion rate of neutralizing antibody 30 days after the full immunization of each group before the immunization, the positive conversion rate of the neutralizing antibody 30 days after the full immunization before the immunization, the positive conversion rate of the neutralizing antibody 30 days after the full immunization, and the full immunization. Neutralizing antibody positive rate after 30 days, S protein binding (IgG) antibody positive rate 30 days after full immunization, and RBD protein binding (IgG) antibody positive rate 30 days after full immunization. The Clopper-Pearson method is used to calculate the 95% confidence interval, and the Chi-square test/ Fisher exact probability method

is used to statistically test the differences between groups.

The statistical analysis of antibody positivity/positivity at 14 days after the first dose, before the second dose, 14 days after the second dose, before the third dose, and 14 days after the third dose is the same as that at 30 days after the whole inoculation.

The levels of IL-2, IL-4, IL-5, IL-6 and IFN-γ are statistically described in each group 4 days after the whole course of vaccination.

The geometric mean and its 95% confidence interval are used to statistically describe the neutralizing antibody GMT and its growth factor 30 days after the full immunization of the negative population before the immunization, and the neutralizing antibody GMT and its growth factor 30 days after the full immunization of the positive population before the immunization, Neutralizing antibody GMT and its growth factor 30 days after full immunization, S protein binding (IgG) antibody GMC and its growth factor 30 days after full immunization, and RBD protein binding (IgG) antibody GMC and its growth factor 30 days after full immunization. And the log-converted anova is used to test the difference between groups statistically.

The statistical analysis of antibody level at 14 days after the first dose, before the second dose, 14 days after the second dose, before the third dose, and 14 days after the third dose is the same as that at 30 days after the whole inoculation.

Drawing the inverse distribution graphs of the titers/concentrations of neutralizing antibodies, S protein binding (IgG) antibodies and RBD protein binding (IgG) antibodies before and after immunization.

12.5.4 Immune Persistence Analysis

Calculate the antibody positive rate of each group at 6 months after full vaccination and 12 months after full vaccination, and use the Chi-square test/Fisher's exact probability method to statistically test the difference between the two groups.

The levels of IL-2, IL-4, IL-5, IL-6 and IFN-γ are statistically described in each group 12 months after the whole course of vaccination.

The geometric mean and its 95% confidence interval are used to statistically describe the antibody levels of each group at 6 months after the full course of vaccination and 12 months after the full course of vaccination and their increase in multiples compared with 30 days after

the full course of vaccination. The variance analysis after logarithmic transformation was used to statistically test the differences between groups.

12.5.5 Safety analysis

MedDRA was used to medically code adverse events and serious adverse events, and classified statistics according to SOC and PT. In addition, according to the provisions of the protocol, the collection of adverse events will be classified and calculated according to systemic reactions and local reactions. This trial mainly performed a statistical analysis of the treatment Emergent Adverse Event (TEAE) that occurred after the first dose of vaccination, and listed the adverse events that occurred before the first dose of vaccination in a list. Unless otherwise specified, the adverse events below are TEAEs.

Calculate all adverse events in each group, adverse events related to the experimental vaccine, adverse events not related to the experimental vaccine, grade 3 and above AEs, grade 3 and above AEs related to the research vaccine, AEs that led to withdrawal, and research The incidence, number and incidence of vaccine-related AEs that led to withdrawal were statistically compared using Fisher's exact probability method. The occurrence, time and severity of adverse events were statistically described. List the list of adverse events related to the experimental vaccine, the list of adverse events not related to the experimental vaccine, and the list of AEs that led to withdrawal.

Calculate the incidence, number and incidence of all serious adverse events in each group, serious adverse events related to the experimental vaccine, and serious adverse events unrelated to the experimental vaccine, and use the Fisher exact probability method to determine the difference between each group Statistical comparison. Make a list of serious adverse events.

12.5.6 Subgroup analysis

This study does not plan to conduct subgroup analysis

12.5.7 Multiplicity problem

This trial is a Phase II immunization program exploratory trial and does not involve multiple issues. In the results of immunogenicity evaluation and safety evaluation, the calculated P value is only the nominal P value, which is mainly used to describe the strength of the association between the evaluation endpoint and the treatment group, not as a process Formal basis for statistical inference.

12.5.8 Processing of missing data

In the statistical analysis of FAS, those with missing serum test results after immunization use the last observation carried forward (LOCF) method for data filling, and further derive the corresponding validity endpoint. This trial no longer deals with missing data in the safety endpoint.

12.5.9 Statistical analysis strategy

The safety results 1 month after inoculation, and the immunogenicity results 1 month after full immunization are analyzed. And write statistical analysis report and summary report.

The safety data, especially serious adverse events, and the immune persistence data for 6 months after the full vaccination were updated and analyzed 6 months after the full vaccination.

After the immune persistence phase is completed, the immune persistence data for 12 months after the full vaccination will be updated and analyzed.

13. Subject safety and adverse event management

13.1 Security precautions

Clinical trials are conducted in medical and health care units which are qualified for vaccination at the county and city level. Sponsors will assess the research site in strict accordance with GCP requirements before the start of the trial, focusing on whether the environmental facilities of the test site meet the requirements of the *Guidelines for Quality Management of Vaccine Clinical Trials (Trial)*, the first aid facilities and first aid equipment in the emergency room are effective, the emergency medicine is within the validity period, and the emergency doctor has the corresponding qualification and ability. When a subject has an adverse event at the test site, he will promptly carry out treatment in the on-site emergency room. If the subject needs urgent hospitalization, after the on-site treatment is stable, the on-site ambulance will send the subject to the agreed hospital for treatment. The test site has signed the *Green Channel Agreement* with the local county-level and above general hospitals, and the subjects should notify the agreed hospitals for timely treatment during the enrollment period. There must be strict SOPs to stipulate personnel responsibilities, contact numbers, rescue routes and other measures to ensure timely handling of unexpected adverse events. Ensure the effective contact between subjects and investigators so that any adverse events can be reported and dealt with quickly. When subjects need to be hospitalized for emergency treatment after a

serious adverse event, the contracted hospital can provide green channel services such as visits, hospitalization, and medical security to ensure that subjects receive timely treatment.

The sponsor appoints full-time personnel to be responsible for the management of clinical trial safety information monitoring and SAE reports (including ADE/VED). Both the sponsor and the researcher should formulate SOP and train all relevant personnel for clinical trial safety information monitoring and SAE reporting (including ADE/VED). The monitoring and reporting of adverse events in vaccine clinical trials is completed by subjects, adverse event investigators, and researchers in stages and at different observation points.

13.2 Discovery and collection of adverse events

Regarding the soliciting and non-solicited AEs of the subjects, the subjects were asked whether they received hospitalization, outpatient treatment, or self-administration for any reason, and recorded this information.

The training of subjects emphasized the need to report adverse events in a timely manner. Researchers should be highly vigilant about such events and investigate and handle them in a timely manner.

When a SAE occurs, the investigator is responsible for reviewing all documents related to the event (such as: hospital history and medical order records, laboratory reports and diagnostic reports), in order to clarify the nature and relevance of SAE, the investigator arranges clinical examination/inspection according to the requirements of the sponsor. If the subject is confirmed dead during the study period or during the follow-up period, the hospital's final conclusions about the deceased should be collected. If an autopsy is performed, a copy of the results, including histopathological results, should be obtained.

Researchers should collect copies of complete cases as much as possible, but cannot replace the study records with copies of subjects' cases. All information related to SAE should be recorded on the original records, eCRF, and serious adverse event report pages.

If medical records should be disclosed for medical identification, all columns that can identify subjects should be obscured before disclosure.

13.3 Serious Adverse Event Report

13.3.1 Report time

After the investigator learns of the SAE, he should immediately report to the sponsor in

writing, and then provide a detailed and written follow-up report in time. The sponsor immediately analyzes and evaluates safety-related information after receiving it, including the severity, correlation with the test drug, and whether it is an expected event, etc.

13.3.2 Report content

1) Report type and report time (first report, follow-up report, final report and corresponding report time);

2) Subject's information (name initials, study number, date of birth, gender);

3) Information of reporter (name of medical institution and professional, telephone number, position/title of reporter);

4) Suspected drug information (Chinese and English drug name, registration classification and dosage form);

5) Research related information (clinical research approval number, clinical research classification, clinical trial indications);

6) Combined disease and treatment information (diagnosis name of disease, name of treatment drug, usage and dosage);

7) Detailed information of SAE (diagnostic name, whether it belongs to ADE/VED, severity criteria, time of occurrence, end time, laboratory test results, treatment process, outcome, measures taken for the test vaccine and the relationship with the test vaccine Relevance, etc.);

8) Blind breaking situation;

9) Time when the investigator learned the information;

10) Signature of the investigator.

13.4 Suspicious and unexpected serious adverse reactions

Suspected Unexpected Serious Adverse Reaction (SUSAR) must meet the following three criteria at the same time: (Extracted from *Regulations on the Management of Reporting of Serious Adverse Events in Vaccine Clinical Trials (Trial)*)

1) Suspected adverse reaction: Refers to the adverse reaction of the subject at any dose that has nothing to do with the purpose of the medication. After analysis, it is considered that the relationship with the drug is at least possibly related.

2) Unexpected adverse reaction: Refers to the nature, extent, consequence or frequency of

the adverse reaction, which is different from the expected risk described in the previous plan or other related materials (such as the investigator's manual). The investigator's manual serves as the main document to provide safety reference information for judging whether an adverse reaction is expected or unexpected.

3) Severe adverse reaction: refers to the severity of the adverse reaction reaching the standard of serious adverse event, and refers to one of the following situations: refers to the death, life-threatening, permanent or severe disability or loss of function or loss of function after the subject receives the experimental drug need to be hospitalized or prolonged, and adverse medical events such as congenital abnormalities or birth defects

According to the nature (category) of the SUSAR event, the sponsor will submit the first report to the Drug Evaluation Center of the National Medical Products Administration and the local provincial drug regulatory authority and notify the clinical trial institution, the main investigator, the ethics committee and the health supervisor according to the following time limit department:

1) For suspicious and unexpected serious adverse reactions (SUSAR) that are fatal or life-threatening, the sponsor should report as soon as possible after the first notification, but not more than 7 natural days, and report relevant follow-up information within the following 8 natural days.

2) For suspicious and unexpected serious adverse reactions (SUSAR) that are not fatal or life-threatening, the sponsor should report as soon as possible after the first notification, but not more than 15 natural days.

3) For other potentially serious security risks, the sponsor should report it as soon as possible after being first notified, but not more than 15 natural days.

At the same time, the rapid report should be carried out in accordance with the specific requirements of the *Standards and Procedures for Rapid Reporting of Safety Data During Drug Clinical Trials*. When the sponsor and the investigator cannot agree on the causality between AE and vaccine, the judgment of either party cannot be ruled out related to experimental vaccines should also be reported quickly.

13.5 Periodic safety reports

During the duration of this trial, the sponsor shall submit regular safety reports to the main investigators of the National Drug Administration Drug Evaluation Center, the local provincial drug regulatory authority, the ethics committee, and the institutions participating in the clinical

trial.

Periodic safety reports are mainly annual reports, submitted in written form according to the requirements of the drug regulatory authority and the ethics committee. The reporting period starts from the date when the vaccine clinical trial is approved and ends when the vaccine is approved for production.

Researchers and sponsors should provide SAE-related information and safety risk assessment reports in a timely manner in accordance with the requirements of the drug regulatory authority and the ethics committee.

The investigator should promptly report the sponsor's safety information report on the clinical trial and submit the annual clinical trial report to the ethics committee.

13.6 Treatment and management of adverse events

Researchers should establish emergency plans for SAE handling in clinical trials, train all relevant personnel, and have measures to be informed of any clinically significant diseases/ events that occur after subjects are vaccinated. And in accordance with the relevant national regulations and current medical management regulations, the subjects should go to the designated hospital for appropriate treatment in time. The drugs used to treat AEs should be recorded in the subject's original record and eCRF.

If disagreements and disputes occur in the handling of adverse events, the investigator is obliged to cooperate with the sponsor in the handling and assist the subjects in medical identification.

The sponsor has the obligation and responsibility to unconditionally guarantee the safety of subjects, and to provide humane care and compensation to subjects who have AEs related to the experimental vaccine during clinical trials.

Investigators should pay continuous attention to AEs that continue due to the termination of AEs or the end of the visit. AEs related to vaccination should be followed up until the end of the event. Follow-ups can be stopped when unrelated events such as diseases are diagnosed by a doctor.

14. Clinical trial management

14.1 Parties and responsibilities of clinical trials

14.1.1 Sponsor

1) Provide the preliminary clinical trial protocol and informed consent, clinical trial related documents such as the final clinical trial protocol, informed consent form, electronic case report form (eCRF) and data management plan approved by review;
2) Provide on-site application documents such as notification of drug clinical trial, investigator's manual (including the chemistry, pharmacy, toxicology, pharmacology, and clinical data and data (including completed and ongoing) of experimental vaccines);
3) Provide trial vaccines for clinical research and issue verification reports;
4) Provide placebo control for clinical research and issue verification report;
5) Responsible for the safe storage and transportation of trial vaccines (including trial vaccine and placebo) and the recovery of remaining test vaccines;
6) Responsible for the safe transportation of serum samples;
7) Designate full-time personnel to be responsible for clinical trial safety information monitoring and SAE report management, to grasp the latest status of the entire clinical trial safety information, and timely report to the relevant parties involved in the trial, the ethics review committee and the regulatory authority, and the suspicious and unexpected serious adverse reactions should also be reported to the health authority;
8) Participate in the investigation and handling of adverse reactions/ events, and be responsible for providing medical or related compensation for adverse reaction cases and clinically proven vaccine-related adverse events according to relevant regulations;
9) Responsible for dispatching qualified inspectors or entrusting a contract research organization to evaluate and select the clinical trial site, perform the inspection duties according to GCP requirements during the trial, and verify the research data;
10) Organize the audit of clinical trials to ensure quality, ensure that clinical trials are conducted in accordance with GCP and program requirements, and bear the ultimate responsibility for the quality of clinical trials;
11) Establish an independent data and security monitoring committee (DSMB);
12) Provide clinical research funding.

14.1.2 Responsible agency

1) Participate in the formulation of clinical trial protocol and organize the implementation of clinical trial plans;

2) Assist in the preparation and review of on-site application forms such as informed consent, vaccination and follow-up records, diary cards, contact cards and case report forms;
3) Submit ethics review materials to the ethics review committee and obtain approval certificates;
4) Establish a vaccine clinical trial organization management system and quality management system, write SOP and conduct training;
5) Recommend the clinical trial site, organize and assist in the standardized construction of the site, and the institution and the on-site "drug clinical trial institution filing management information platform" for filing;
6) Have management mechanisms and measures to prevent and deal with emergencies in vaccine clinical trials, have a team of SAE emergency response experts and technical capabilities to deal with SAE;
7) Responsible for the epidemic safety training of clinical trial personnel;
8) Ensure the safe storage and use of test vaccines, and manage biological samples;
9) Organize on-site recruitment, enroll subjects, organize on-site vaccination, and supervise the implementation of on-site work;
10) Organize and complete the entry of all forms and electronic case report forms (eCRF) at the test site;
11) Organize the follow-up of subjects, the collection of adverse events and pregnancy events at the test site, and organize the investigation, handling and reporting of adverse events and pregnancy events;
12) Issue a summary report of clinical trials.

14.1.3 Trial site

1) Establish a team of qualified on-site researchers, establish environmental facilities that meet the requirements of clinical trials, and assist the responsible agency in filing drug clinical trials;
2) Recruit and enroll subjects who meet the requirements of the clinical trial protocol;
3) Complete vaccination, sample collection and safety follow-up observation;
4) Deal with adverse events, deviations and events that violate the plan during the study, and report serious adverse events, deviations and events that violate the plan according to regulations;
5) Collect the original data of clinical trials and enter them into eCRF;
6) Manage test vaccines and biological samples in accordance with GCP requirements;

7) Accept the inspection, inspection and on-site verification by a third party (the sponsor, the national bureau, and the provincial bureau);

8) In accordance with GCP requirements, manage and save trial-related data used for drug registration applications until 5 years after the vaccine is approved for marketing.

14.1.4 Data and Security Monitoring Board (DSMB)

DSMB responsibilities:

Set up DSMB to monitor the safety data during the study period.

1) DSMB is composed of 3 experts (1 each for clinical medicine, epidemiology and statistics).

2) DSMB is responsible for monitoring the safety data during the study period.

3) The safety data of all subjects in the 3-dose group after 30 days was reviewed by DSMB experts.

4) If a suspension/termination criteria event occurs, the DSMB convenes an emergency meeting to evaluate whether to terminate the trial, and promptly report to the ethics committee and the National Drug Evaluation Department.

5) In the case of severe or dead cases after the novel crown infection that occurred during the trial, the DSMB will convene an emergency meeting to conduct safety assessments and organize special investigations. If the results suggest the presence of ADE/VED phenomenon, it will be immediately reported to the ethics committee and the National Drug Evaluation Department.

14.1.5 Technical Cooperation Unit

1) Complete the blind detection of clinical trial samples and issue a test report;

2) Test methods use pharmacopoeial methods or test kits that have been approved for marketing by the country, and provide reference values for result determination, test standards and test standards, and provide method verification certificates when necessary; test methods such as those provided by the vaccine developer The provider is responsible for the methodological verification and provides relevant data for the method verification of the drug efficacy testing unit.

3) Issue relevant laboratory qualification certificates for certification, accreditation and quality control.

14.1.6 Statistical Unit

1) Responsible for the randomization, sample size and statistical analysis of the clinical trial protocol;

2) Write a statistical analysis plan based on the clinical trial plan;

3) Random and blinded clinical trials;
4) Perform statistical analysis according to the planned statistical analysis plan and write statistical analysis reports.

14.1.7 Data Management Unit

1) Complete the eCRF draft and amendments according to the requirements of the plan, and review and finalize the draft jointly by researchers, sponsors, statisticians, project managers, etc.;
2) Develop a data management plan and data verification plan according to the plan and eCRF;
3) Work on database creation, testing, revision, database backup, version upgrade and transmission according to SOP;
4) Perform data cleanup according to the data verification plan, which mainly includes: check for missing data, check against the plan, time window check, logic check, scope check, SAE consistency check, etc.; issue an online question, and the researcher will clarify;
5) Carry out data quality control inspection;
6) Responsible for the medical coding of this study, the coding content includes non-solicited AE (including SAE)
7) Responsible for the classification of combined drugs in this study;
8) After the clarification of the data question is completed, complete the data review report; sort out the statistical population division resolution according to the opinions of the researcher, sponsor, statistician, etc.; lock the database, and deliver the data to the statistician for statistical analysis;
9) Complete the data management report;
10) After the project is completed, the XPT format data set and the relevant registration documents for data management shall be engraved on the CD and submitted to the sponsor.

14.1.8 Contract Research Organization (CRO)

Carry out clinical trial monitoring based on GCP, clinical trial protocol, and SOP:

1) Assist the sponsor to confirm that the clinical trial institution undertaking the trial has the appropriate conditions to complete the trial, including staffing and training, functional areas such as emergency rooms, and the laboratories are well-equipped and functioning well, and have various conditions related to the trial;

2) Verify that the experimental vaccine is transported, stored, distributed, used, returned and processed according to the requirements of the protocol during the entire test process, and controlled and recorded, and check the dose change and combined medication of each subject;
3) Confirm that all subjects have signed written informed consent before the test, and confirm that the selected subjects are qualified;
4) Confirm that the investigator has received the latest version of the investigator's manual, protocol, all clinical trial related documents, and test supplies, and implemented it normally in accordance with the requirements of regulations;
5) Verify that the researchers have been trained and obtained written authorization before participating in the research;
6) Confirm that all data records and reports are correct and complete, all eCRF entries are correct and consistent with the original data, and verify that all medical reports, records and documents provided by the investigator are accurate, complete, timely, clear and easy to read, date and research Number, verify that the corrections, additions or deletions made to the data are correct, dated, and signed by the researcher;
7) Confirm that all adverse events are recorded, and serious adverse events are reported and recorded within the specified time;
8) Make sure that the researchers keep the necessary documents in accordance with the GCP requirements, and the test records and documents are updated in real time and kept intact;
9) Determine the deviation from the trial protocol, SOP, GCP and relevant regulatory requirements in clinical trials, communicate with the investigator in time, and take appropriate measures to prevent the deviation from recurring;
10) Verify that the withdrawal and loss of the selected subjects have been stated in the eCRF;
11) The inspector shall send a written report to the sponsor after each inspection, and explain the corrective measures that have been taken or planned for the problems found in the inspection; truthfully record the follow-up that the investigator failed to achieve , Tests and inspections not performed, and whether errors and omissions are

corrected;

12) After the completion of the clinical trial, assist the research institution and the sponsor to organize the documents and submit it to the National Medical Products Administration (NMPA), and assist in the preparation for the registration on-site verification.

14.2 Site management

This clinical trial is a single-center clinical trial, and the responsible agency is the Provincial Center for Disease Control and Prevention.

The Provincial Center for Disease Control and Prevention has vaccination management functions and a team of investigators, with professional departments, and the central laboratory is accredited by the national laboratory. Responsible for appointing the main investigators of clinical trials for clinical trial management, setting up clinical trial coordinators, quality controllers, adverse event investigators and clinical trial-related personnel. The main investigator is responsible for interpreting the clinical research plan, organizing GCP and SOP training, and the coordinator and quality controller for the management and quality control of the entire clinical trial process.

The on-site Center for Disease Control and Prevention is a subordinate unit that accepts business management/ guidance from provincial institutions. It has the function of implementing vaccination, participates in clinical trials under the leadership of the responsible institution, and has on-site directors, on-site investigators, quality controllers, and adverse event investigators. And other positions, according to requirements to carry out vaccination, observation and follow-up, adverse event reporting and handling.

15. Quality assurance and monitoring of clinical trials

15.1 Investigator

The responsible agency for vaccine clinical trials should establish a complete organizational management system and quality management system, have management mechanisms and measures to prevent and deal with emergencies in vaccine clinical trials, have SAE emergency response expert teams and technical capabilities for handling serious adverse events, It has complete cold chain equipment for vaccine delivery and storage.

The trial site for vaccine clinical trials has the qualifications for vaccination approved by the

administrative department of health, has a relatively fixed and sufficient number of clinical trial researchers, is equipped with standard operating procedures related to vaccine clinical trials, and has training and training records. Medical institutions cooperate to establish a green channel for SAE medical treatment of vaccine clinical trials. According to the vaccination and visit process of vaccine clinical trials, there are reception area, informed consent room, consultation and physical examination screening room, biological specimen collection room, vaccination room, emergency room, medical observation room, vaccine storage room, and archives room. , Sample processing and preservation rooms, case screening laboratories and temporary storage places for medical wastes, etc., establish first-aid green channels, and have ambulances and related rescue personnel and first-aid items on the test site.

The division of labor of all researchers must be confirmed by the main researchers to ensure that all researchers participating in this project have the corresponding qualifications, are trained and authorized, clarify their respective tasks, and master and implement relevant standard operating procedures. Researchers in the responsible institutions and test sites have been trained on GCP and vaccine clinical trial technology, and have training records. Supporting personnel should have records of participating in corresponding work training.

15.2 Sponsor

The sponsor is ultimately responsible for the quality of clinical trials. A complete vaccine clinical trial quality management system should be established, corresponding SOPs should be formulated, clinical trial audits should be organized, and clinical trial related activities and documents should be systematically checked, including trial sites, laboratories, CRO companies, etc., to evaluate whether the trial is in compliance with the trial protocol, SOP and relevant laws and regulations require that the test data are recorded in a timely, true, accurate and complete manner. Audits are performed by personnel not directly involved in clinical trials.

The trial site should cooperate with the audit of clinical trial projects, keep relevant records, formulate improvement plans for problems found in the audit, and adopt corresponding management measures to improve the quality of the trial.

15.3 Monitor

According to Article 50 of the *Guiding Principles for the Quality Management of Vaccine Clinical Trials (Trial)*, the sponsor shall appoint a sufficient number of inspectors to monitor the entire clinical trial. The inspector should have medical, pharmacy or related professional education background and work experience. The number of monitors designated by the sponsor for clinical trials of vaccines should be determined based on the frequency of monitoring the

trial and the complexity of the trial plan design. The inspector shall conduct the inspection of clinical trials in accordance with the requirements of the inspection plan and submit an inspection report.

The monitor supervises the entire clinical trial process to ensure that the implementation of the clinical trial complies with the trial protocol, SOP, GCP, and relevant regulatory requirements, and is completed within expectations.

15.4 Biological sample management

The samples used for laboratory inspection (if applicable) are managed by a dedicated person and sent to the testing laboratory at room temperature to track the samples until the test is completed.

It is used for immunogenic blood samples, managed by a dedicated person, and establishes sample storage and temperature records. The storage temperature needs to be monitored and recorded daily on working days (under the premise of automatic temperature monitoring and alarm, holidays can be arranged according to the specific situation on the spot) monitoring and Record the temperature.

Serum and plasma samples should be sent to the testing laboratory under freezing (dry ice/ low-temperature ice row/other freezing methods). Backup samples should not be shipped at the same time as the submitted samples. The investigator is responsible for proper preservation (≤ -20°C) After the clinical report is completed, it will be processed after confirmation by the sponsor.

Lymphocyte samples should be sent to the laboratory of China Food and Drug Control Institute for immunological function cell or cytokine-related testing under the condition of ≤ -150 ℃.

All types of sample transfer management should be strictly managed throughout the entire process. Researchers, sample transporters, and laboratories should keep proper records.

15.5 Vaccine management

The responsible agency for vaccine clinical trials shall guide the trial site to formulate a management system for experimental vaccines, and the management of the receipt, storage, recovery, return/destroy of experimental vaccines shall meet the requirements of relevant laws and regulations. The vaccine clinical trial agency and the trial site shall designate personnel

who have passed GCP and relevant training to be responsible for the management of trial vaccines.

Vaccine delivery: The whole process of vaccine management must meet the cold chain requirements, and there must be vaccine transportation and storage conditions that meet the requirements of the plan. The vaccine should be stored and transported at 2-8°C and protected from light. The vaccine delivery process must have a delivery note and temperature monitoring. The packaging and unpacking temperature should be recorded upon arrival. After receiving the vaccine, the recipient will sign and fax or copy the delivery note to The shipper, both parties properly keep the delivery note.

Vaccine storage, distribution and use: test vaccines are stored in separate areas at 2-8°C and protected from light, and managed by special personnel and special counters. Blind management should be maintained for blind trials. Vaccine recipients must verify vaccine delivery status, establish vaccine transfer, registration, use, and recovery work forms, fill them out as required, and keep them in work records.

Vaccine handover record: The sponsor will provide the test vaccine, and the researcher will check the name, quantity, and packaging of the vaccine while receiving the vaccine, and make a handover record.

Vaccine registration and use records: The researcher establishes vaccine registration and use records. The vaccine distributed to each subject should be used in a record, including the study number, the initials of the subject, the signature of the person who vaccinated, etc.

Vaccine recovery records: Vaccine administrators should collect the remaining vaccines in a timely manner, place them separately, conduct regular counts and complete the count records. If the vaccine usage and remaining quantity do not match the total number, the situation should be explained. The discarded, expired, and remaining vaccines in this trial are returned to the sponsor. All outsourced equipment for the used vaccines must be retained for inspection during the trial. The sponsor will check the number of vaccines while receiving the vaccine, make relevant records, and sign by the vaccine administrator and the representative of the sponsor. The sponsor must keep the vaccine in accordance with the cold chain requirements at least until the NMPA verification is completed.

Cold chain destruction: Once an abnormality of < 2°C or > 8°C occurs in the

temperature of the stored vaccine, it is regarded as cold chain destruction. The sponsor should state information about vaccine stability in the investigator's handbook. Once cold chain damage occurs, the investigator should transport the vaccine to a dark environment at 2~8℃, suspend the use of cold chain damaged vaccine, and report to the sponsor as soon as possible , According to the written opinion of the sponsor to decide to stop or continue to use the vaccine. Vaccines with cold chain destruction cannot be used for subjects until the sponsor's advice is received.

Trial vaccines and control vaccines are not allowed to be used in non-clinical trial populations.

15.6 Equipment calibration

- The refrigerator has been validated, temperature monitoring has been implemented, and it is used within the validity period, and there must be 3 consecutive days of normal temperature monitoring records before use;

- The refrigeration equipment used to store vaccines and samples on site has been inspected annually and is in use;

- The thermometer has been standardized;

- Vaccine syringes are disposable sterile syringes; venipuncture needles and vacuum blood collection tubes are disposable sterile supplies. The manufacturer has a national production license and records the batch number and validity period;

- The centrifuge can operate normally and has daily maintenance records;

- Sphygmomanometers, height gauges, weight scales and other measuring instruments are qualified for use.

15.7 Original data management

Informed consent, logbook for vaccination and follow-up, diary cards, contact cards, SAE report forms and other original materials are important basis for clinical trial traceability. They should be recorded in a timely, accurate, complete, standardized and true manner, and properly kept on the research site.

Authorized and specially trained researchers enter the research data into the EDC based on the original data. The entry should not be changed at will. If the entry is incorrect, it should be modified in accordance with the filling guide. In order to ensure the authenticity of clinical

trial data, the inspector and the investigator will jointly review the EDC. After the investigator has signed, all data will be processed by the clinical trial responsible unit or the sponsor entrusted by the statistician.

15.8 Research materials

The sponsor and the investigator provide clinical trial data in accordance with the *Administrative Measures for Drug Registration* and GCP.

The investigator folder is organized according to GCP requirements and stored in the research center. The research center is responsible for collating and summarizing the materials delivered to the sponsor. Materials that record the true information of subjects, such as screening registration form, informed consent, vaccination and follow-up records, diary cards, contact cards, subject medical records, etc., are sealed in the research center, and the coordinator of the responsible institution and the on-site archivist will do To check the handover, both parties sign a deposit agreement or memorandum.

File management is carried out in accordance with SOP, with identification signs including the name of the project, completion date, sponsor, and retention period, and security measures such as insect prevention, moisture prevention, fire prevention, and theft prevention. The use and access of the project data is limited to the relevant personnel of the project, the relevant personnel of the sponsor (including the project inspector) and the NMPA project inspector. All materials used to apply for drug registration will be kept until 5 years after the vaccine is approved for marketing, and the sponsor will be notified after the expiration date. No one may dispose of it without authorization until the sponsor is notified in writing.

15.9 Agreement and clinical summary report

The responsibilities of all parties to the clinical trial are stipulated in the agreement, which will become effective after the parties concerned sign and seal. The original agreement is in 4 copies, and the sponsor and researcher have 2 copies each.

Clinical summary report (red chapter): 5 copies, 1 copy for the investigator, 4 copies for the sponsor to declare the new drug certificate and approval number, and the copy can be kept at the research site.

16. The Ethics Committee

16.1 Review clinical trials

The ethics committee needs to review the scientific and ethical rationality of drug clinical trial projects, aiming to ensure the dignity, safety and rights of subjects, promote the scientific and healthy development of drug clinical trials, and enhance public trust and support for drug clinical trials.

The ethics committee can review the trial protocol, informed consent form, recruitment materials, and other written materials provided to the subjects. The revision of the protocol needs to be negotiated with the sponsor. If the content of the informed consent form does not violate the protocol and conforms to local conditions. The situation can adopt the opinion of the ethics committee.

16.2 Implementation process review

16.2.1 Follow-up review

The ethics committee shall follow up and review all approved clinical trials until the end of the trial.

16.2.2 Review of amendments

Any modification of the trial protocol during the clinical trial process should be submitted to the ethics committee for review and approval/recording before implementation. The ethics committee should request the sponsor and/or investigator to submit relevant information for review of the amendment, including (but not limited to):

(1) The content of the modification and the reason for the modification;

(2) The impact of the revised plan on the expected risks and benefits;

(3) The impact of the revised program on the rights and safety of subjects.

The ethics committee mainly evaluates the trial risks and benefits of the modified protocol and makes review opinions. In order to avoid causing emergency harm to the subjects, the researcher can implement it before submitting it to the ethics committee for review and approval, and make a written report to the ethics committee afterwards.

16.2.3 Annual/regular follow-up review

During the initial review, the ethics committee should determine the frequency of annual/periodic follow-up review based on the degree of risk of the trial, at least once a year. The ethics committee should require researchers to submit reports on time. The annual/regular

follow-up review report information includes (but is not limited to):

(1) The progress of the trial;

(2) Number of subjects included, number of completed cases, number of withdrawal cases, etc.;

(3) Confirm that serious adverse events are reported in time and handled properly;

(4) Any events or new information that may affect the benefits of research risks;

After reviewing the research progress, the ethics committee re-evaluated the risks and benefits of the trial.

16.2.4 Review of serious adverse events

The ethics committee needs to review the serious adverse events reported by the sponsor and/ or investigator, including the extent and scope of the serious adverse event, the impact on the trial risk benefit, and the medical protection measures for the subjects.

16.2.5 Non-compliance/violation of protocol review

For non-compliance/ violation of the protocol during the clinical trial, the ethics committee should ask the sponsor and/or investigator to explain the cause, impact and handling measures of the incident, and examine whether the incident affects the safety and rights of subjects , Whether it affects the risk and benefit of the experiment.

16.2.6 Early termination of trial review

If the sponsor and/or investigator terminate the trial early and need to report to the ethics committee for review, the ethics committee should request the sponsor and/or investigator to report the reason for the early termination of the trial, as well as the follow-up treatment of the subjects, and review the safety of the subjects And whether the rights are guaranteed.

16.2.7Final review

The ethics committee should require the sponsor and/ or investigator to report the completion of the trial and review the safety and protection of the rights and interests of the subjects.

17. Study team

The responsible institution of clinical trial
The institution name: Hunan Provincial Center for Disease Control and Prevention Address: No. 450, Section 1, Furong Middle Road, Changsha

Postal Code: 41005

The person in charge: Fangjun Li

Phone: 13574109585

Fax: 0731-84305903

E-mail: 646022285@qq.com

Professional department: the center of vaccine clinical research

The responsible party: Fangjun Li

Phone: 0731-84305935

Fax: 0731-84305903

E-mail: 646022285@ qq.com

Clinical trial site

The site name: Xiangtan County Center for Disease Control and Prevention

Address: No. 228, Haitang Road, Yisuhe Town, Xiangtan County

Postal Code: 411228

The person in charge: Tingdong Yan

Phone: 13975216152

Fax: 0731-52582079

E-mail: cdcytd@126.com

Data and Safety Monitoring Board (DSMB)

The Chairman:

Name: Jielai Xia

Unit: Department of Health Statistics, Department of Military Preventive Medicine, Fourth Military Medical University

Phone: 029-84774804

E-mail: 13571999716@163.com

Committee expert Ⅰ:

Name: Xuanyi Wang

Unit: Institute of Biomedicine, Fudan University

Phone: 021-54237865

E-mail：xywang@shmu.edu.cn

Committee expert Ⅱ:

Name: Dongliang Yang

Unit: Department of Infectious Diseases, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology Phone: 027-85726130 E-mail: dlyang@hust.edu.cn
Technical Cooperation Unit
Unit name: The National Institutes for Food and Drug Control Professional department: Hepatitis enterovirus vaccine department Address: No. 31, Huatuo Road, Daxing District, Beijing Postal Code: 102629 Person in charge: Zhongyu Hu Phone: 010-53851772 E-Mai: huzy67@163.com
Statistical unit
Unit: Beijing Kangterike Statistical Technology Co., Ltd. Address: Room 1018w, Sihui Building, Chaoyang District, Beijing Post Code: 100023 Person in charge: Zhiwei Jiang Phone: 18618483152 E-mail: zhi.wei.jiang@ktstat.com
Data management unit
Unit: Beijing Kangterike Statistical Technology Co., Ltd. Address: Room 1018w, Sihui Building, Huihe South Street, Chaoyang District, Beijing Post Code: 100023 Person in charge: Guijuan Li Phone: 13601034495 E-mai: gui.juan.li@ktstat.com
Contract Research Organization (CRO)
Company name: Chongqing Medleader Biomedical Co., Ltd. Address: No.669, Maliu Avenue, Mudong Town, Banan District, Chongqing Post code: 401338

Project leader: Yan Liu
Phone: 023-68179162
E-mail: liuyan@medleader.com.cn

18. Publication of papers

After the trial is over, the research unit can publish the summary report or research results related to the clinical trial in the form of a paper after obtaining the written authorization of the sponsor, and the investigators in research units and technical collaboration units (pharmacodynamic evaluation) have the right to sign papers. The research results that are negative or inconclusive should be published or made public the same as positive results.

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Appendix Ⅰ: Introduction to clinical trial institutions and site conditions

The responsible institution of clinical trial

The Hunan Provincial Center for Disease Control and Prevention was established in March 2001. It was formed by the merger and reorganization of the former Hunan Provincial Health and Epidemic Prevention Station, Hunan Provincial Health Education Institute, and Hunan Provincial Institute of Dermatology Prevention and Treatment. It is subordinate to the Hunan Provincial Health Commission. A public welfare institution organized by the Hunan Provincial Government to implement disease prevention and control and public health technical management and services. It is the province’s disease prevention and control, health testing and inspection, health education, preventive medical research, preventive technology consulting and services, and it is also a disease Control organization's business leadership, technical assessment and professional training center. The center has gathered a large number of high- and intermediate-level health professional and technical personnel, and has a large number of advanced equipment. There are high-level laboratories certified by the World Health Organization and the country, such as AIDS confirmation laboratory, polio laboratory, disinfection identification laboratory, sanitary insecticide registration and efficacy laboratory, and health food functional inspection and evaluation experiment. The ISO9001 quality management system has been established, and it has the strong ability to provide disease prevention and treatment technical services for the whole province and promote the overall development of the province's health and disease prevention.

Since the implementation of planned immunization in Hunan Province in 1978, the whole province has established a relatively complete immunization planning service network and vaccine management system. The development of immunization planning talent team, vaccine cold chain system construction, vaccination information management, vaccination rate monitoring and We have accumulated a wealth of experience in the monitoring of abnormal vaccination reactions. The Hunan Provincial Center for Disease Control and Prevention has established a department specifically responsible for vaccine clinical research. At present, it has established a relatively complete vaccine clinical trial quality control and assurance system. It has carried out measles vaccine, rubella vaccine, influenza A H1N1 vaccine, varicella vaccine, four Phase Ⅰ, Ⅱ, and Ⅲ clinical trials of multiple vaccines including high-valent influenza vaccine, HPV vaccine, herpes zoster vaccine, Hib conjugate vaccine, rotavirus vaccine, and rabies vaccine.

The clinical trial site

Xiangtan County is located in the east of central Hunan Province, on the west bank of the middle and lower reaches of the Xiangjiang River. It has a total area of 2132.8 square kilometers, jurisdiction over 17 towns, 645 administrative villages, a total population of 980,000, and a permanent population of 853,200.

Xiangtan County Center for Disease Control and Prevention was established on December 1, 2004 on the basis of the original Xiangtan County Health and Epidemic Prevention Station. It is a technical service guidance center for disease prevention and control and public health inspection and inspection in Xiangtan County. The unit covers an area of 8,125 square meters. The building area is 6456 square meters, the inspection room is 2485 square meters, the office building is 3971 square meters, and there are 90 employees. Among them, 54 health professionals (5 associate high, 28 intermediate, 21 junior). There are 3 functional departments and 9 business departments, including office, quality personnel department, finance department, immunization planning department, emergency health emergency and infectious disease prevention and control department (internal vector control department), tuberculosis Department of AIDS Prevention and Control, Department of School Health, Department of Public Health, Prevention and Control of Chronic Non-communicable Diseases, Department of Laboratory Medicine, Department of Health Education and Health Promotion (internal science and education department), project office, etc.

Xiangtan County Center for Disease Control and Prevention is the practice teaching base of Central South University, the vaccine clinical test base and the field research base of health food efficacy observation of the Hunan Provincial Center for Disease Control and Prevention, the "Epidemiology Research Base" of the Institute of Pathogenic Biology, Chinese Academy of Medical Sciences, Hunan Province The first batch of county-level A-level disease control centers established the only county-level network virus laboratory in the province. From 2015 to 2016, the center successfully completed the "Influenza Virus Split Vaccine Clinical Trial" project with 1,200 volunteers inoculated. From 2017 to 2018, the project of the “Phase III Clinical Trial of Herpes Zoster Vaccine” with 6,000 volunteers inoculated was carried out. Since 2019, the project of "Phase III clinical trial of oral hexavalent reassortant rotavirus live vaccine" with 1100 volunteers inoculated has been carried out.

Appendix Ⅱ: Personnel authorized by the sponsor to revise the trial protocol

Name	Position	Unit
Yilin Wang	Senior Medical Writer	Chongqing Medleader Biomedical Co., Ltd.