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Supplementary appendix 4

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

Supplement to: Zeng G, Wu Q, Pan H, et al. Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. *Lancet Infect Dis* 2021; published online Dec 7. https://doi.org/10.1016/S1473-3099(21)00681-2.

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Supplemental Methods

Exclusion criteria for the administration of the second and third dose

- **Continuing vaccination is prohibited, and other research steps may continue according to researchers' judgement if:**

  (1) A COVID-19 vaccine other than the experimental vaccine was used during the study period;

  (2) Any serious adverse reaction with causality assessed as vaccine caused;

  (3) Severe anaphylaxis or hypersensitivity after vaccination (including urticaria / rash within 30 minutes after vaccination);

  (4) Any confirmed or suspected autoimmune or immunodeficiency disease, including human immunodeficiency virus (HIV) infection;

- **Vaccination can be postponed within the time window specified in the program if:**

  (5) Acute or new chronic disease occurrs after vaccination;

  (6) Other reactions (including severe pain, severe swelling, severe activity limitation, persistent high fever, severe headache or other systemic or local reactions) as judged by the investigator;

- **Vaccination can be postponed within the time window specified in the protocol if:**

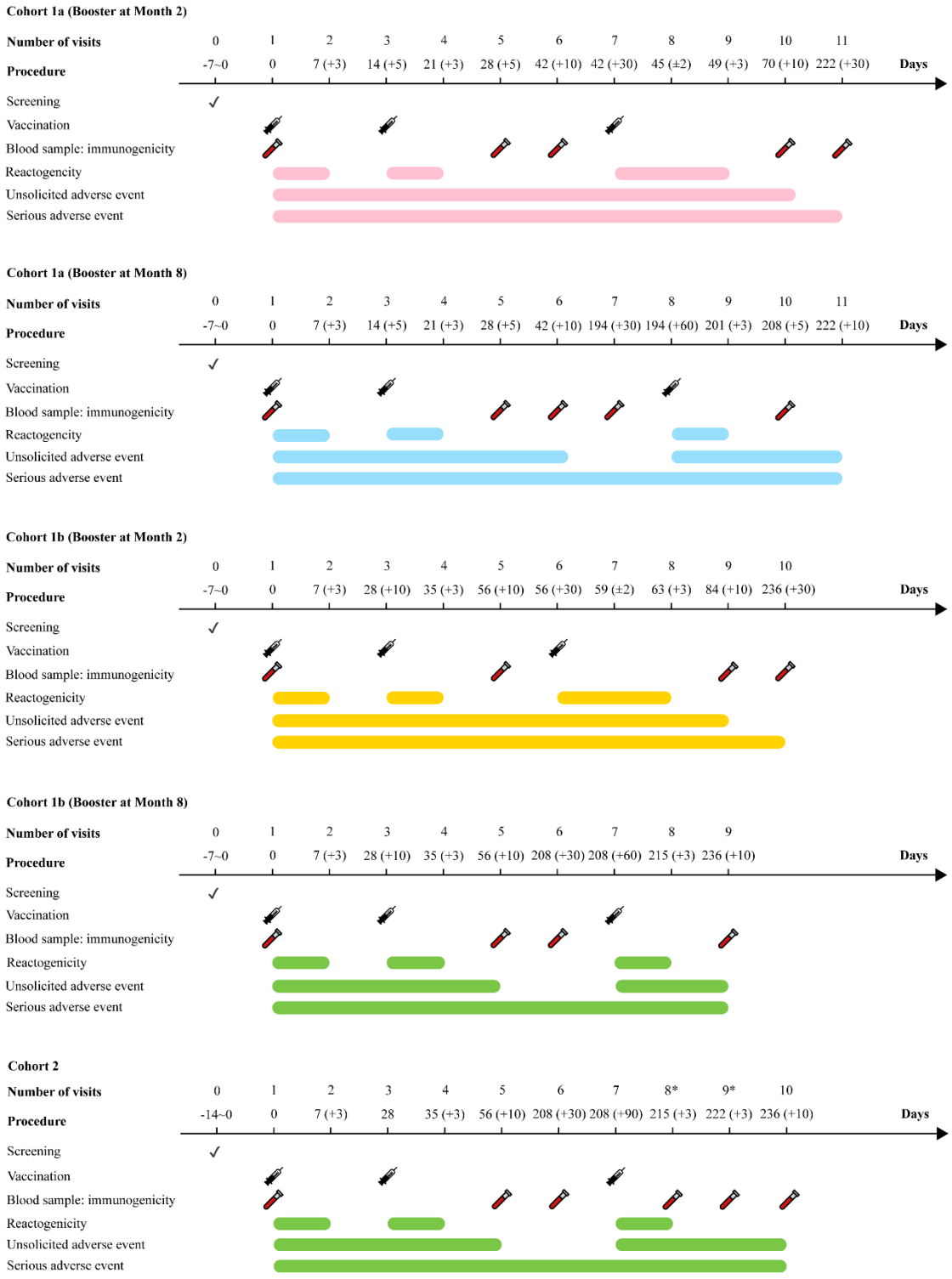
  (7) At the time of vaccination, the participant was suffering from acute disease (acute disease refers to moderate or severe disease with or without fever);

  (8) The axillary temperature was higher than 37.0℃;

  (9) Participants were vaccinated with a (non-Covid-19) subunit vaccine or inactivated vaccine within 7 days or a live, attenuated vaccine within 14 days;

  (10) According to the judgment of the investigator, the participant had any factors not suitable for vaccination.

Trial profile in the protocol



Note: The time window for each visit specified in the protocol is indicated in brackets.

In ***Cohort 1a***, routine hematological and biochemical tests were performed for the first 30 participants before dose 3 and within 3 days after dose 3. Participants would not be assigned a third dose if the severity of hematology and biochemistry indexes was grade 2 or more.

Detection Method of Neutralization Potency against Live SARS-CoV-2

Micro cytopathic effect assay was adopted.

Serum treatment: all serum samples were inactivated at 56°C in a water bath for 30 minutes.

Medium addition: the cell maintenance medium was added to the cell control group at 100 μL/well, and 50 μL/well of maintenance medium was supplemented to the to-be-tested serum group, virus back titration group and positive control group from the second dilution.

Dilution of the serum sample: The serum was diluted four-fold (60 μL sample + 180 μL maintenance medium) with cell maintenance medium (2% newborn calf serum-199 (2% sodium hydrogen carbonate) cell maintenance medium). The diluted serum was added to the cell plate at 100 μL/well, and each sample was diluted to 2 wells in parallel. 50 μL of the mixture in the first dilution was pipetted into the next dilution, and the mixture was pipetted up and down for 8-10 times. The mixture was diluted to the appropriate dilution range by this method, and 50 μL of the last dilution was discarded, and 50 μL of the diluted sample was retained in each well.

Dilution of the virus for neutralization: the SAR-CoV-2 used for neutralization was diluted to 100$CCID_{50}$/0.05ml by titer.

Neutralization: Serum of different dilutions was mixed with 100CCID50/0.05ml virus liquid in equal volume (50 μL+50 μL), respectively, and then incubated in an incubator at 36.5°C, 5%CO_2 for 2h.

Experimental control: Negative serum control, positive serum control, serum sample and cell control were set simultaneously.

Virus Back Titration: The virus suspension diluted to 100 CCID50/0.05 mL was diluted via ten-fold serial dilution, i.e. diluted to 10 CCID50/0.05 mL, 1 CCID50/0.05 mL and 0.1 $CCID_{50}$/0.05 mL, and added to the 96-well cell plate respectively, 12 well per dilution and 50 μL per well, then 50 μL of cell maintenance medium was added to each well, and the plate was incubated in an incubator at 36.5°C, 5% CO2 for 5 days. Cell Inoculation and Culture: After incubation, 100μL of Vero cell suspension (cell concentration: 1.0-2.0×10^5 cell/mL) was added to each well, and then incubated in an incubator at 36.5°C, 5% CO2 for 5 days.

Interpretation of the Results: It was observed for the cytopathic effect after cultured for 3-5 days, and the neutralizing antibody titer of the to-be-tested serum sample was determined according to the observation results of the cytopathic effect (CPE). The reciprocal of the highest serum dilution without cytopathic effects the end titer. When 1 of the 2 wells of the highest dilution serum shows CPE, while the other does not, the reciprocal of the dilution should be the neutralizing antibody titre of the serum specimen; the reciprocal of the mean dilution of the two wells should be the neutralizing antibody titre of the serum specimen when the 2 wells with the highest dilution are completely pathological while the adjacent 2 wells with low dilution are not pathological completely; when 1 of two adjacent wells is pathological while the other not, the reciprocal of the average dilutions of 2 wells should be the neutralizing antibody titer of the serum specimen. For example, 2 wells with high dilution of 1:8 have a complete CPE, while the adjacent 2 wells with low dilution of 1:16 have no CPE; or in 2 adjacent wells with dilutions of 1:8 and 1:16, one has a CPE, while the other does not. In this case, the reciprocal 12 of the average dilutions of 2 wells is the neutralizing antibody titer of the serum.

Study endpoints list

Phase 2 trial among adults aged 18-59 years old

Primary endpoint

- Positive conversion rate of serum neutralizing antibody on Day 14 (***Cohort 1***)/Day 28 (***Cohort 2***) after two doses of test vaccine;
- Incidence of adverse reaction on Day 0-28 (Day 0-14 for the first dose for ***Cohort 1***) after each dose;

Secondary endpoint

- Positive rate, GMT and GMI of serum neutralizing antibody on Day 28 after two doses of test vaccine;
- Positive conversion rate, positive rate, GMT and GMI of serum neutralizing antibody on Day 28 after three doses of test vaccine (only for ***Cohort 1a-14d-2m*** and ***Cohort 2a-28d-2m***);
- Incidence of adverse reactions 0-7 days after each dose of vaccination;
- Incidence of serious adverse event from the inoculation to 6 months after full course vaccination;

Exploratory Endpoints

- The seropositive rate and GMT of neutralizing antibody at 6 months after the second dose (only for ***Cohort 1b-14d-8m*** and ***Cohort 2b-28d-8m***);
- The seropositive rate, GMT, and GMI of neutralizing antibody at 12 months after the third dose (only for ***Cohort 1a-14d-2m*** and ***Cohort 2a-28d-2m***);
- The seropositive rate and GMT of neutralizing antibody at 14 days (only for ***Cohort 1b-14d-8m***) or 28 days (only for ***Cohort 2b-28d-8m***) after the booster dose;
- The seropositive rate and GMT of neutralizing antibody at 6 months after the booster (only for ***Cohort 1b-14d-8m*** and ***Cohort 2b-28d-8m***).

Phase 2 trial among adults aged 60 years and older

Primary Endpoint

- Incidence of adverse reactions within 28 days after each dose of vaccination;
- The seroconversion rate of neutralizing antibodies 28 days after the second dose vaccination.

Secondary Endpoint

- Incidence of adverse reactions within 7 days after each dose vaccination;
- Incidence of SAEs from the beginning of the vaccination to 12 months after booster immunization;
- The seropositive rate, GMT, and GMI of neutralizing antibodies 28 days after the second dose vaccination;

Exploratory Endpoint

- The seropositive rate and GMT 6 months after the second dose vaccination;
- The seropositive rate, GMT, and GMI 28 days after the booster vaccination;
- The seropositive rate and GMT 6 months after the booster vaccination;
- The seropositive rate and GMT 12 months after the booster vaccination.

Members of Independent Data Monitoring Committee

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Fujie Zhang	Clinical Medicine	Beijing Ditan Hospital

Descriptions of retest results for the older adults

A range of measures were taken to control the quality of neutralization tests in our study as follows:

Back titration: Due to the SARS-CoV-2 was an unprecedented virus emerged in the world, standardized neutralization test process has not been established for SARS-CoV-2. As one of the most commonly used and the most reliable methods for quantifying antibodies against a selected virus, neutralization tests are the gold-standard assay for the poliovirus. Based on the standardize virus neutralization methods to measure antibody levels against poliovirus in human sera released by WHO [Polio laboratory manual 4th edition, 2004. World Health Organization, Geneva, Switzerland], we conducted the back titration for confirming the amount of virus used in each test was within the range 32 - 320 $TCID_{50}$/50 µl.

Positive antibody control: Two kinds of positive antibody control were set up for each time of our experiment, the antibody titer value of positive control used between batches are stable and reasonable. The positive antibody controls were selected as anti-goat antibody (titre range: 1024-4096) and human serum (XGR, titre range: 64-256). Anti-goat antibody was generated by immunizing goats with purified SARS-CoV-2. Human serum was generated from convalescent plasma of COVID-19 patients.

Negative antibody control: Negative antibody control were set up for each time of our experiment, the antibody titer value of negative control used between batches are stable and reasonable. The negative serum control was selected as rat serum. Rat serum was obtained from non-immunized rats by centrifugation.

Serum toxicity control: Serum toxicity was determined by incubation of the lowest serum dilution (1:2) with cells without virus. Serum toxicity control was set for each sample.

Cell control: Cell control wells comprising cells alone were set up for each time of our

experiment.

Immunogenicity of CoronaVac was evaluated in younger adults and older adults, separately. Four vaccination schedules were assessed in the younger adults (18-59 years old) and one vaccination schedule was assessed in the older adults (aged 60 years and older). The immunogenicity was evaluated by neutralizing antibodies against SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank number MT407649.1) which were quantified using a micro-cytopathogenic effect assay.

Specimens obtained on day 28 after the second dose had been tested previously, and the neutralizing antibody titres were comparable between younger adults (18 to 59 years of age) and older adults (60 years of age or above). However, neutralizing antibody titres of sera obtained on day 28 after the third dose from older adults was approximately two-fold higher (***Cohort 3 b-28d-8m***) compared with titres from younger adults (***Cohort 2b-28d-8m***) who had been immunized with the same vaccination schedules [GMT: 143.1 (95%CI 110.8-184.7) in the younger adults, 342.8 (95%CI 266.4-441.1) in the older adults], which was in contrast with the pattern of other vaccines and the knowledge of young adults with more powerful immune response capacity. To verify the stability and reliability of neutralizing antibody test results, we retested convenience samples of specimens from 100 younger adults and 100 older adults. Pairwise comparison was conducted between neutralization results of initial-testing and re-testing and results were shown below:

Group	**Test**	**GMT (95%CI)**	**Pairwise group t-test (P value)**	**Cohen's kappa test (p value)**
Younger adults	Initial-test	77.8 (51.4-117.7)	0.76	0.68
	Re-test	71.1 (47.8-105.9)		
Older adults	Initial-test	146.8 (99.2-217.4)	0.02	<0.001
	Re-test	77.3 (53.8-111.2)		

For younger adults, the neutralizing antibody titres were similar with these two tests. Accordingly, the results of the first test for this population were used in our analysis. For older adults, the neutralizing antibody titre were significantly lower in the re-tests. We evaluated our protocol and data recording steps to ensure data correctness. The evaluation showed procedures consistent with the protocol. It is not possible to judge with certainty which result is more reliable.

In addition, the comparison results of demographics of the total participants included in the initial immunogenicity and selected participants were shown as below, which indicated that there were no significant differences. Considering the acceptable results of serum samples between younger adults and older adults in the retests, we used the retest results of 100 older adults, which we believe to be more reliable, in our analyses. Due to repeated freezing and thawing, and insufficient sera, we were unable to retest specimens from the other older adults. Excluding two participants who were vaccinated mistakenly, there were a total of 98 participants included in the immunogenicity analysis of third dose in older age group.

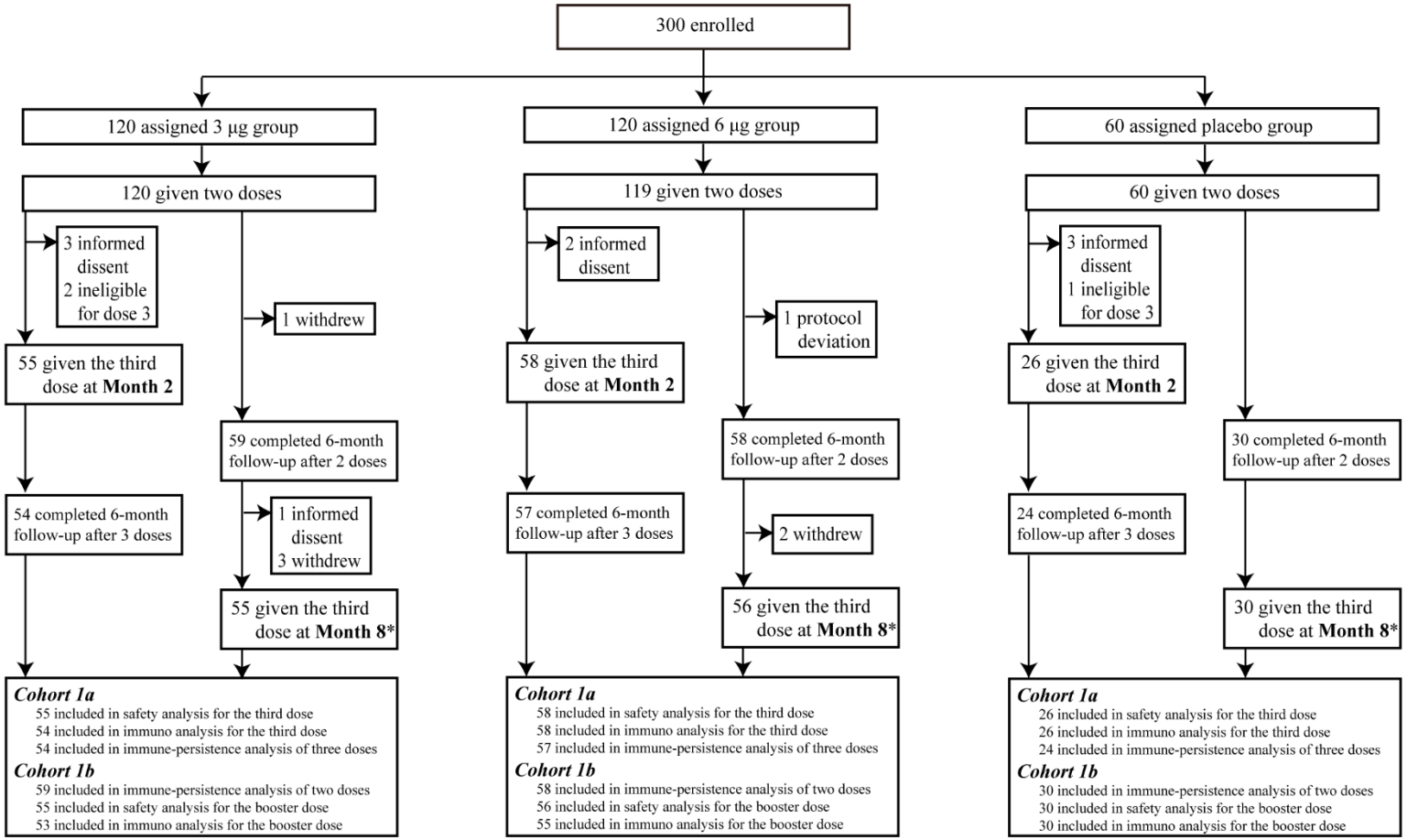
	Total	**Sample**	**P value**
1.5 μg			
N	82	28	—
Age (Mean±SD)	66.3±4.4	66.3±4.5	0.98
Sex (Male/Female)	44/38	14/14	0.91
3 μg			
N	87	29	—
Age (Mean±SD)	66.4±4.4	67.2±4.9	0.49
Sex (Male/Female)	46/41	14/15	0.83
6 μg			
N	78	28	—
Age (Mean±SD)	66.3±4.4	67.0±5.2	0.57
Sex (Male/Female)	42/36	16/12	0.94
Placebo			
N	42	13	—
Age (Mean±SD)	67.2±4.8	67.0±4.5	0.91
Sex (Male/Female)	17/25	4/9	0.76

Vaccination intervals between the second dose and third dose

	No of participants received the third dose	Actual interval and corresponding number of participants	No of participants received the third dose out of window (protocol violation)	Mean	Median	IQR	Range
Cohort 1a-14d-2m	139	47: 25 55: 1 56: 113	0	54.4	56	56-56	47-56
Cohort 1b-14d-8m	141	249: 16 250: 103 251: 22	141 (9-11 days)	250.0	250	250-250	249-251
Cohort 2a-28d-2m	130	38: 1 40: 22 49: 4 51: 103	0	49.0	52	51-51	38-51
Cohort 2b-28d-8m	130	235: 11 236: 24 237:3 238: 92	0	237.4	238	236-238	235-238
Cohort 3-28d-8m	303	240: 1 247: 1 248: 2 255: 123 256: 154 257: 21 258: 1	0	255.5	256	255-256	240-258

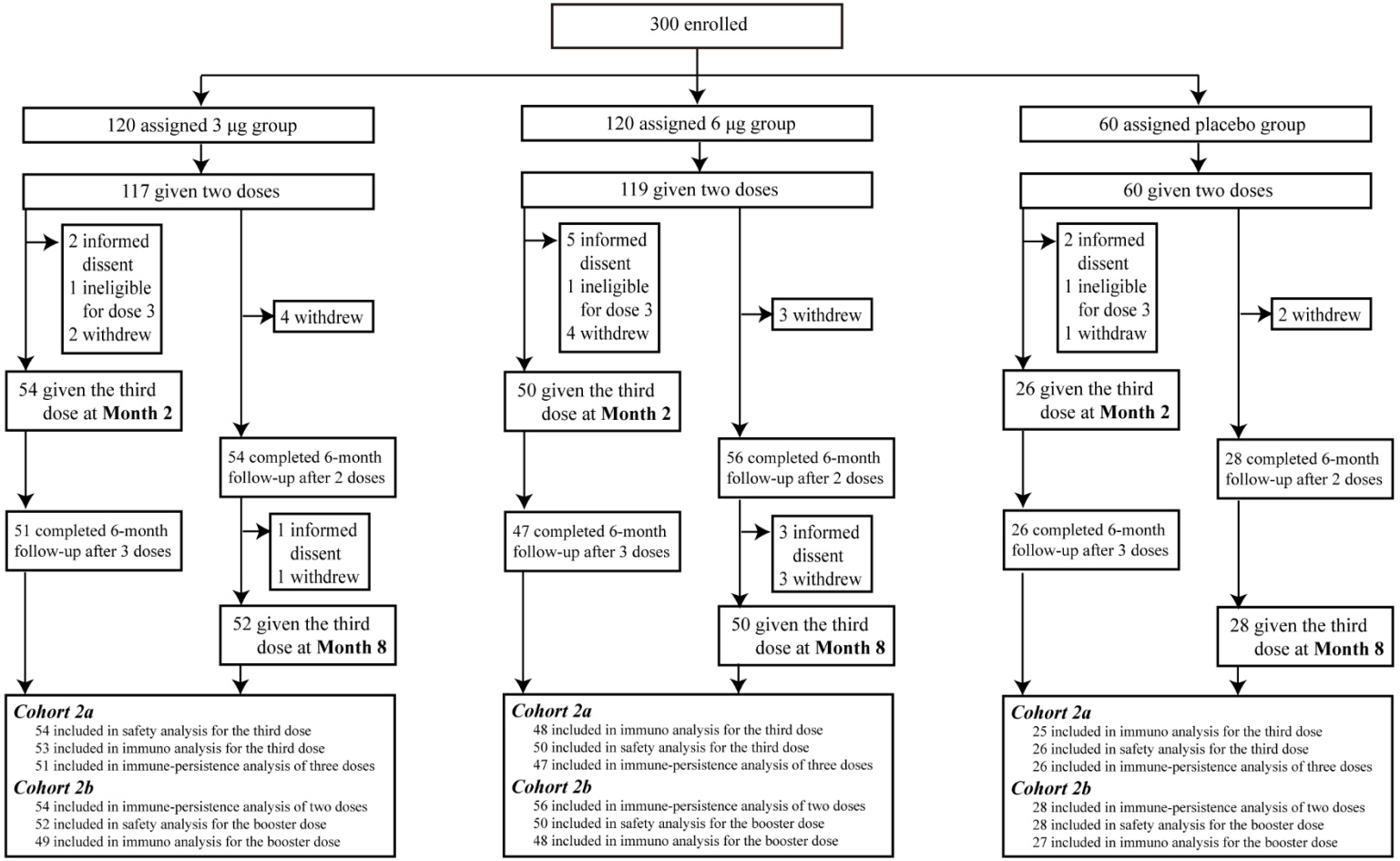
Supplemental Flowcharts

A. Cohort 1, adults aged 18-59 years old

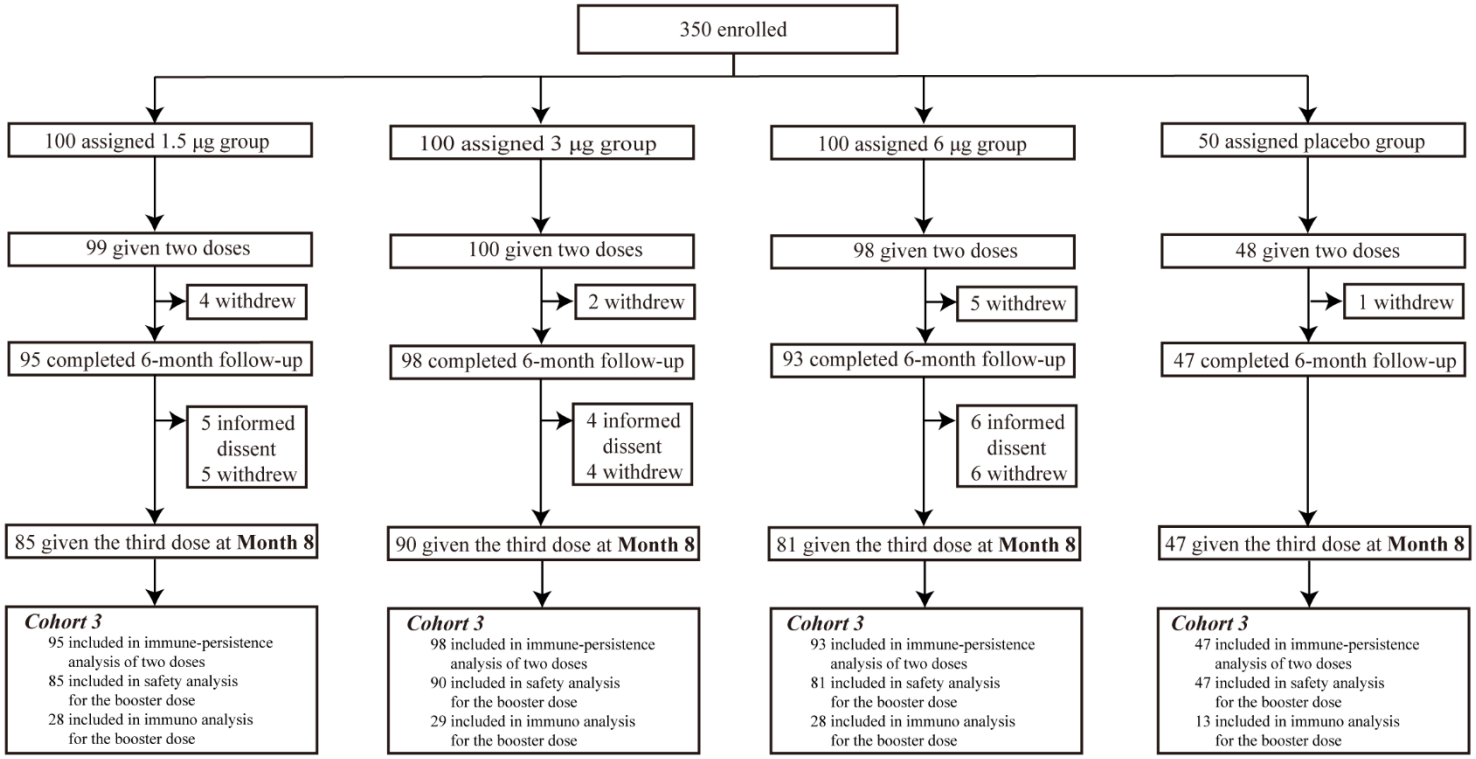


*Participants received the third dose between day 249 and day 251, outside the time window specified in the protocol.

B. Cohort 2, adults aged 18-59 years old



C. Cohort 3, adults aged 60 years and older



Supplemental Results of Immunogenicity

Table S1. Six-month immune persistence after primary three-dose regimen of CoronaVac in adults (immune-persistence analysis set)

Indicator		3 μg group	6 μg group	placebo group	P value*	P value†
Cohort 1a-14d-2m						
Baseline (Pre-immunization)						
	Seropositivity [n/N, %, (95%CI)]	0/54, 0 (0.00-6.60)	0/57, 0 (0.00-6.27)	0/24, 0 (0.00-14.25)	1.00	—
	GMT (95%CI)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NA	—
Day 28 after dose 2						
	Seropositivity [n/N, %, (95%CI)]	50/54, 93 (82.11-97.94)	56/57, 98 (90.61-99.96)	0/24, 0 (0.00-14.25)	<0.01	—
	GMT (95%CI)	21.8 (17.3-27.6)	29.5 (23.8-36.6)	2.0 (2.0-2.0)	<0.01	—
Day 28 after dose 3						
	Seropositivity [n/N, %, (95%CI)]	53/54, 98 (90.11-99.95)	56/57, 98 (90.61-99.96)	0/24, 0 (0.00-14.25)	<0.01	1.00
	GMT (95%CI)	45.8 (35.7-58.9)	73.5 (58.3-92.7)	2.0 (2.0-2.0)	<0.01	<0.01
Month 6 after dose 3						
	Seropositivity [n/N, %, (95%CI)]	33/54, 61 (46.88-74.08)	39/57, 68 (54.76-80.09)	0/24, 0 (0.00-14.25)	<0.01	0.42
	GMT (95%CI)	9.2 (7.1-12.0)	13.6 (10.5-17.7)	2.1 (2.0-2.3)	<0.01	0.04

Cohort 2a-28d-2m						
Baseline (Pre-immunization)						
	Seropositivity [n/N, %, (95%CI)]	0/51, 0 (0.00-6.98)	0/47, 0 (0.00-7.55)	0/26, 0 (0.00-13.23)	1.00	—
	GMT (95%CI)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NA	—
Day 28 after dose 2						
	Seropositivity [n/N, %, (95%CI)]	48/51, 94 (83.76-98.77)	47/47,100 (93.80-100.00)	0/26, 0 (0.00-13.23)	<0.01	—
	GMT (95%CI)	37.1 (27.4-50.2)	56.5 (43.6-73.2)	2.0 (2.0-2.0)	<0.01	—
Day 28 after dose 3						
	Seropositivity [n/N, %, (95%CI)]	50/51, 98 (89.55-99.95)	47/47,100 (93.80-100.00)	0/26, 0 (0.00-13.23)	<0.01	1.00
	GMT (95%CI)	48.8 (38.9-61.3)	51.0 (40.5-64.2)	2.0 (2.0-2.0)	<0.01	0.78
Month 6 after dose 3						
	Seropositivity [n/N, %, (95%CI)]	34/51, 67 (52.08-79.24)	24/47, 51 (36.06-65.92)	0/26, 0 (0.00-13.23)	<0.01	0.12
	GMT (95%CI)	10.0 (7.3-13.7)	10.2 (7.1-14.6)	2.0 (2.0-2.0)	<0.01	0.95

GMT (geometric mean titer) was calculated based on log-transformation data. Seropositivity threshold was set as a titer of 8.
ANOVA model with log-transformation (GMT) was used to detect the difference among groups. Pairwise comparison between groups was conducted by group t-test with log-transformation. P value was calculated by Fisher exact probability method and of the comparison of seropositivity rate. Bonferroni correction was performed as a post hoc test.
p* values are for comparisons among all groups. p† values are for comparisons between 3 μg group and 6 μg group.

Table S2. Six-month immune persistence after two doses of CoronaVac in adults (immune-persistence analysis set)

	Indicator	3 μg group	6 μg group	placebo	P value*	P value†
Cohort 1b_14d/8m						
Baseline (Pre-immunization)						
	Seropositivity [n/N, %, (95%CI)]	0/59, 0 (0.00-6.06)	0/58, 0 (0.00-6.16)	0/30, 0 (0.00-11.57)	1.00	—
	GMT (95%CI)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	NA	—
Day 28 after dose 2						
	Seropositivity [n/N, %, (95%CI)]	56/59, 95 (85.85-98.94)	58/58,100 (93.80-100.00)	0/30, 0 (0.00-11.57)	<0.01	—
	GMT (95%CI)	26.2 (21.3-32.3)	30.7 (25.0-37.7)	2.0 (2.0-2.0)	<0.01	—
Month 6 after dose 2						
	Seropositivity [n/N, %, (95%CI)]	10/59, 17 (8.44-28.97)	14/58, 24 (13.87-37.17)	0/30, 0 (0.00-11.57)	<0.01	0.34
	GMT (95%CI)	4.1 (3.3-5.2)	4.8 (3.8-6.1)	2.0 (2.0-2.0)	<0.01	0.36
Cohort 2b_28d/8m						
Baseline (Pre-immunization)						
	Seropositivity [n/N, %, (95%CI)]	0/54, 0 (0.00-6.60)	0/56, 0 (0.00-6.38)	0/28, 0 (0.00-12.34)	1.00	—

	GMT (95%CI)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	2.0 (2.0-2.0)	1.00	—
Day 28 after dose 2						
	Seropositivity [n/N, %, (95%CI)]	54/54, 100 (93.40-100.00)	56/56, 100 (93.62-100.00)	0/28, 0 (0.00-12.34)	<0.01	—
	GMT (95%CI)	48.4 (39.0-60.1)	71.7 (58.4-87.9)	2.1 (1.9-2.3)	<0.01	—
Month 6 after dose 2						
	Seropositivity [n/N, %, (95%CI)]	19/54, 35 (22.68-49.38)	26/56, 46 (32.99-60.26)	0/28, 0 (0.00-12.34)	<0.01	0.23
	GMT (95%CI)	6.7 (5.2-8.6)	7.1 (5.6-8.9)	2.0 (2.0-2.0)	<0.01	0.76

GMT (geometric mean titer) was calculated based on log-transformation data. Seropositivity threshold was set as a titer of 8.
ANOVA model with log-transformation (GMT) was used to detect the difference among groups. Pairwise comparison between groups was conducted by group t-test with log-transformation. P value was calculated by Fisher exact probability method and of the comparison of seropositivity rate. Bonferroni correction was performed as a post hoc test.
p* values are for comparisons among all groups. p† values are for comparisons between 3 μg group and 6 μg group.

Table S3. Six-month immune persistence after two doses of CoronaVac in older adults (immune-persistence analysis set)

Indicator		1.5 μg group	3 μg group	6 μg group	placebo	P value			
						Among four groups	1.5 μg group vs 3 μg group	1.5 μg group vs 6 μg group	3 μg group vs 6 μg group
Baseline (Pre-immunization)									
	Seropositivity [n/N, %, (95%CI)]	0/95, 0 (0.00-3.81)	0/98, 0 (0.00-3.89)	1/93, 1 (0.03-5.85)	0/47, 0 (0.00-7.55)	0.42	—	—	—
	GMT (95%CI)	2.0 (2.0-2.0)	2.0 (2.0-2.1)	2.1 (1.9-2.2)	2.0 (2.0-2.0)	0.60	—	—	—
Day 28 after dose 2									
	Seropositivity [n/N, %, (95%CI)]	86/95, 91 (82.78-95.58)	95/98, 97 (91.31-99.36)	93/93, 100 (96.11, 100.00)	0/47, 0 (0.00-7.55)	<0.01	—	—	—
	GMT (95%CI)	23.0 (19.0-27.8)	40.8 (33.8-49.3)	50.5 (42.7-59.8)	2.1 (2.0-2.1)	<0.01	—	—	—
Month 6 after dose 2									
	Seropositivity [n/N, %, (95%CI)]	12/95, 13 (6.70, 21.03)	17/98, 17 (10.44-26.31)	21/93, 23 (14.55-32.42)	1/47, 2 (0.05-11.29)	0.01	0.36	0.07	0.37
	GMT (95%CI)	3.1 (2.7-3.6)	3.4 (2.9-4.1)	4.1 (3.3-5.0)	2.1 (1.9-2.2)	<0.01	0.41	0.04	0.22

GMT (geometric mean titer) was calculated based on log-transformation data. Seropositivity threshold was set as a titer of 8.
ANOVA model with log-transformation (GMT) was used to detect the difference among groups. Pairwise comparison between groups was conducted by group t-test with log-transformation. P value was calculated by Fisher exact probability method and of the comparison of seropositivity rate. Bonferroni correction was performed as a post hoc test.

Table S4. Comparison of neutralising antibody levels induced by dose 3 among the four groups in adults aged 18-59 years old (cohorts 1 and 2)

Variable	Coefficient	Statistics	P-value
Intercept	5.45	19.49	<0.001
Age	-0.03	-5.07	<0.001
Sex			
Male	Reference group		
Female	0.12	1.22	0.22
Dose group			
3 µg	Reference group		
6 µg	0.49	2.61	0.009
Schedule group			
Cohort 1a-14d-2m	Reference group		
Cohort 1b-14d-8m	0.70	3.63	<0.001
Cohort 2a-28d-2m	0.43	2.24	0.04
Cohort 2b-28d-8m	1.33	6.92	<0.001
Sampling date			
Day 28 after dose 2	Reference group		
Day 28 after dose 3	1.34	17.07	<0.001
Cross reaction			
6 µg: Cohort 1b-14d-8m	-0.11	-0.40	0.69
6 µg: Cohort 2a-28d-2m	-0.16	-0.59	0.55
6 µg: Cohort 2b-28d-8m	0.06	0.23	0.82

Supplemental Results of safety

Table S5. Adverse reactions reported within 28 days post each dose for Cohort 1a-14d-2m

Adverse Reactions (System organ class, preferred term)	Dose 1†					Dose 2					Dose 3				
	3 μg group (N=60)	6 μg group (N=60)	Placebo (N=30)	Total (N=150)	P value*	3 μg group (N=60)	6 μg group (N=60)	Placebo (N=30)	Total (N=150)	P value*	3 μg group (N=55)	6 μg group (N=58)	Placebo (N=26)	Total (N=139)	P value*
Total	11(18%)	11(18%)	5(17%)	27(18%)	1.00	9(15%)	10(17%)	1(3%)	20(13%)	0.20	5(9%)	6(10%)	-	11(7%)	0.25
Grade 1	11(18%)	11(18%)	5(17%)	27(18%)	1.00	9(15%)	10(17%)	1(3%)	20(13%)	0.20	5(9%)	5(9%)	-	10(7%)	0.35
Grade 2	1(2%)	-	-	1(1%)	1.00	1(2%)	1(2%)	-	2(1%)	1.00	1(2%)	1(2%)	-	2(1%)	1.00
General disorders and administration site conditions	7(12%)	10(17%)	4(13%)	21(14%)	0.75	9(15%)	9(15%)	-	18(12%)	0.05	3(5%)	5(9%)	-	8(6%)	0.40
Injection-site pain	5(8%)	8(13%)	3(10%)	16(11%)	0.70	7(12%)	8(13%)	-	15(10%)	0.09	3(5%)	5(9%)	-	8(6%)	0.40
Fatigue	1(2%)	1(2%)	2(7%)	4(3%)	0.33	2(3%)	-	-	2(1%)	0.36	-	-	-	-	-
Fever	1(2%)	1(2%)	-	2(1%)	1.00	-	-	-	-	-	-	-	-	-	-
Injection-site swelling	-	-	-	-	-	-	2(3%)	-	2(1%)	0.36	-	-	-	-	-
Injection-site hypoesthesia	1(2%)	1(2%)	-	2(1%)	1.00	-	-	-	-	-	-	-	-	-	-
Injection-site redness	-	-	-	-	-	1(2%)	1(2%)	-	2(1%)	1.00	-	-	-	-	-
Injection-site discoloration	-	-	-	-	-	1(2%)	-	-	1(1%)	1.00	-	-	-	-	-
Injection-site itching	-	-	-	-	-	1(2%)	-	-	1(1%)	1.00	-	-	-	-	-
Injection-site induration	-	-	-	-	-	-	1(2%)	-	1(1%)	1.00	-	-	-	-	-
Gastrointestinal disorders	3(5%)	-	-	3(2%)	0.23	-	-	1(3%)	1(1%)	0.20	1(2%)	1(2%)	-	2(1%)	1.00
Diarrhea	-	-	-	-	-	-	-	-	-	-	1(2%)	-	-	1(1%)	0.58
Nausea	1(2%)	-	-	1(1%)	1.00	-	-	-	-	-	1(2%)	1(2%)	-	2(1%)	1.00
Vomiting	1(2%)	-	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-

Adverse Reactions (System organ class, preferred term)	Dose 1†					Dose 2					Dose 3				
	3 μg group (N=60)	6 μg group (N=60)	Placebo (N=30)	Total (N=150)	P value*	3 μg group (N=60)	6 μg group (N=60)	Placebo (N=30)	Total (N=150)	P value*	3 μg group (N=55)	6 μg group (N=58)	Placebo (N=26)	Total (N=139)	P value*
Nervous system disorders	1(2%)	-	1(3%)	2(1%)	0.68	1(2%)	1(2%)	-	2(1%)	1.00	-	1(2%)	-	1(1%)	1.00
Headache	-	-	1(3%)	1(1%)	0.20	1(2%)	1(2%)	-	2(1%)	1.00	-	1(2%)	-	1(1%)	1.00
Dizziness	1(2%)	-	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-
Musculoskeletal and connective tissue disorders	2(3%)	-	-	2(1%)	0.36	-	-	-	-	-	1(2%)	-	-	1(1%)	0.58
Muscle pain	2(3%)	-	-	2(1%)	0.36	-	-	-	-	-	1(2%)	-	-	1(1%)	0.58
Respiratory, thoracic and mediastinal disorders	-	1(2%)	-	1(1%)	1.00	-	-	-	-	-	-	1(2%)	-	1(1%)	1.00
Cough	-	1(2%)	-	1(1%)	1.00	-	-	-	-	-	-	1(2%)	-	1(1%)	1.00

†: Reported within 14 days post dose 1.

P value***** was calculated by Fisher exact probability method and of the comparison of incidence rate among three groups.

Table S6. Adverse reactions reported within 28 days after the third dose for Cohort 1b-14d-8m

Adverse Reactions (System organ class, preferred term)	3 μg group (N=55)	6 μg group (N=56)	Placebo (N=30)	Total (N=141)	P value *
Total	10(18%)	13(23%)	3(10%)	26(18%)	0.33
Grade 1	10(18%)	13(23%)	3(10%)	26(18%)	0.33
Grade 2	1(2%)	0(0%)	1(3%)	2(1%)	0.52
General disorders and administration site conditions	8(15%)	11(20%)	2(7%)	21(15%)	0.30
Injection-site pain	8(1%)	9(16%)	0(0%)	17(12%)	0.05
Injection-site itching	0(0%)	1(2%)	2(7%)	3(2%)	0.11
Fever	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Fatigue	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Injection-site swelling	0(0%)	0(0%)	1(3%)	1(1%)	0.21
Nervous system disorders	1(2%)	2(4%)	1(3%)	4(3%)	1.00
Headache	1(2%)	2(4%)	1(3%)	4(3%)	1.00
Dizziness	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Respiratory, thoracic and mediastinal disorders	1(2%)	2(4%)	0(0%)	3(2%)	0.80
Cough	0(0%)	2(4%)	0(0%)	2(1%)	0.35
Laryngeal stimulation	1(2%)	0(0%)	0(0%)	1(1%)	0.60
Oropharyngeal pain	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Musculoskeletal and connective tissue disorders	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Muscle pain	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Gastrointestinal disorders	1(2%)	0(0%)	0(0%)	1(1%)	0.60
Nausea	1(2%)	0(0%)	0(0%)	1(1%)	0.60
Eye disorders	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Periorbital oedema	0(0%)	1(2%)	0(0%)	1(1%)	1.00

*P value was calculated by Fisher exact probability method and of the comparison of incidence rate among three groups.

Table S7. Adverse reactions reported within 28 days post each dose for Cohort 2a-28d-2m

Adverse Reactions (System organ class, preferred term)	Dose 1					Dose 2					Dose 3				
	3 μg group (N=60)	6 μg group (N=60)	Placebo (N=30)	Total (N=150)	P value*	3 μg group (N=59)	6 μg group (N=60)	Placebo (N=30)	Total (N=149)	P value*	3 μg group (N=54)	6 μg group (N=50)	Placebo (N=26)	Total (N=130)	P value*
Total	11(18%)	10(17%)	7(23%)	28(19%)	0.74	3(5%)	6(10%)	2(7%)	11(7%)	0.67	3(6%)	1(2%)	-	4(3%)	0.53
Grade 1	11(18%)	9(15%)	7(23%)	27(18%)	0.58	3(5%)	6(10%)	2(7%)	11(7%)	0.67	3(6%)	1(2%)	-	4(3%)	0.53
Grade 2	-	2(3%)	-	2(1%)	0.36	-	-	-	-	-	-	-	-	-	-
General disorders and administration site conditions	8(13%)	10(17%)	5(17%)	23(15%)	0.87	2(3%)	6(10%)	2(7%)	10(7%)	0.40	2(4%)	1(2%)	-	3(2%)	1.00
Injection-site pain	7(12%)	6(10%)	3(10%)	16(11%)	1.00	2(3%)	4(7%)	2(7%)	8(5%)	0.71	1(2%)	1(2%)	-	2(2%)	1.00
Fatigue	1(2%)	2(3%)	1(3%)	4(3%)	1.00	-	1(2%)	-	1(1%)	1.00	1(2%)	-	-	1(1%)	1.00
Fever	-	3(5%)	-	3(2%)	0.23	-	2(3%)	-	2(1%)	0.52	-	-	-	-	-
Injection-site discoloration	-	-	1(3%)	1(1%)	0.20	-	-	-	-	-	-	-	-	-	-
Injection-site swelling	-	-	-	-	-	-	-	1(3%)	1(1%)	0.20	-	-	-	-	-
Injection-site redness	-	1(2%)	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-
Musculoskeletal and connective tissue disorders	2(3%)	1(2%)	1(3%)	4(3%)	1.00	-	-	-	-	-	-	-	-	-	-
Muscle pain	2(3%)	1(2%)	1(3%)	4(3%)	1.00	-	-	-	-	-	-	-	-	-	-
Gastrointestinal disorders	1(2%)	-	1(3%)	2(1%)	0.68	1(2%)	1(2%)	-	2(1%)	1.00	1(2%)	-	-	1(1%)	1.00
Diarrhea	1(2%)	-	-	1(1%)	1.00	1(2%)	-	-	1(1%)	0.60	1(2%)	-	-	1(1%)	1.00
Nausea	-	-	-	-	-	-	-	-	-	-	1(2%)	-	-	1(1%)	1.00
Vomiting	-	-	1(3%)	1(1%)	0.20	-	1(2%)	0(0%)	1(1%)	1.00	-	-	-	-	-
Metabolism and nutrition disorders	-	1(2%)	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-

Adverse Reactions (System organ class, preferred term)	Dose 1					Dose 2					Dose 3				
	3 µg group (N=60)	6 µg group (N=60)	Placebo (N=30)	Total (N=150)	P value*	3 µg group (N=59)	6 µg group (N=60)	Placebo (N=30)	Total (N=149)	P value*	3 µg group (N=54)	6 µg group (N=50)	Placebo (N=26)	Total (N=130)	P value*
Decreased appetite	-	1(2%)	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-
Respiratory, thoracic and mediastinal disorders	-	-	-	-	-	-	-	-	-	-	2(4%)	-	-	2(2%)	0.68
Cough	-	-	-	-	-	-	-	-	-	-	2(4%)	-	-	2(2%)	0.68
Nervous system disorders	-	1(2%)	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-
Headache	-	1(2%)	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-
Immune system disorders	1(2%)	-	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-
Hypersensitivity	1(2%)	-	-	1(1%)	1.00	-	-	-	-	-	-	-	-	-	-

P value* was calculated by Fisher exact probability method and of the comparison of incidence rate among three groups.

Table S8. Adverse reactions reported within 28 days after the third dose for Cohort 2b-28d-8m

Adverse Reactions (System organ class, preferred term)	3 μg group (N=52)	6 μg group (N=50)	Placebo group (N=28)	Total (N=130)	P value*
Any adverse reaction					
Grade 1	7(13%)	10(20%)	1(4%)	18(14%)	0.13
Grade 2	1(2%)	1(2%)	1(4%)	3(2%)	1
Systemic diseases and injection site adverse reactions					
Injection site pain					
Grade 1	6(12%)	7(14%)	0(0%)	13(10%)	0.11
Injection site swelling					
Grade 1	1(2%)	0(0%)	0(0%)	1(1%)	1
Injection site itch					
Grade 1	1(2%)	0(0%)	0(0%)	1(1%)	1
Fever					
Grade 1	0(0%)	1(2%)	0(0%)	1(1%)	0.6
Grade 2	1(2%)	0(0%)	1(4%)	2(2%)	0.69
Fatigue					
Grade 1	1(2%)	2(4%)	0(0%)	3(2%)	0.61
Respiratory, thoracic and mediastinal disorders					
Runny nose					
Grade 1	0(0%)	1(2%)	0(0%)	1(1%)	0.6
Oropharyngeal pain					
Grade 2	1(2%)	0(0%)	0(0%)	1(1%)	1
Nervous system disorders					
Dizziness					
Grade 1	—	—	—	—	—
Headache					
Grade 1	1(2%)	1(2%)	1(4%)	3(2%)	1
Gastrointestinal disorders					
Diarrhea					
Grade 1	1(2%)	0(0%)	0(0%)	1(1%)	1
Nausea					
Grade 1	0(0%)	2(4%)	0(0%)	2(2%)	0.19
Musculoskeletal and connective tissue disorders					
Myalgia					
Grade 2	0(0%)	1(2%)	0(0%)	1(1%)	0.6

*P value was calculated by Fisher exact probability method and of the comparison of incidence rate among three groups.

Table S9. Adverse reactions reported within 28 days after the third dose for Cohort 3-28d-8m

Adverse Reactions (System organ class, preferred term)	1.5 μg group (N=85)	3 μg group (N=90)	6 μg group (N=81)	Placebo group (N=47)	Total (N=303)	P value*
Any adverse reaction						
Grade 1	3(4%)	3(3%)	3(4%)	2(4%)	11(4%)	1
Grade 2	1(1%)	2(2%)	2(2%)	1(2%)	6(2%)	0.95
Systemic diseases and injection site adverse reactions						
Injection site pain						
Grade 1	1(1%)	2(2%)	2(2%)	1(2%)	6(2%)	0.95
Injection site erythema						
Grade 2	0(0%)	1(1%)	0(0%)	0(0%)	1(0%)	1
Fatigue						
Grade 2	0(0%)	1(1%)	0(0%)	1(2%)	2(1%)	0.52
Respiratory, thoracic and mediastinal disorders						
Cough						
Grade 1	1(1%)	0(0%)	0(0%)	1(2%)	2(1%)	0.34
Grade 2	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Runny nose						
Grade 1	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Nervous system disorders						
Dizziness						
Grade 1	1(1%)	1(1%)	0(0%)	0(0%)	2(1%)	1
Headache						
Grade 1	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Gastrointestinal disorders						
Nausea						
Grade 1	1(1%)	1(1%)	0(0%)	0(0%)	2(1%)	1
Musculoskeletal and connective tissue disorders						
Myalgia						
Grade 2	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.7
Skin and subcutaneous tissue disorders						
Rash						
Grade 2	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42

*P value was calculated by Fisher exact probability method and of the comparison of incidence rate among three groups.

Table S10. Serious adverse events reported in adults aged 18-59 years old

Adverse events (MedDRA 23.0)	3 μg group	6 μg group	Placebo	Total	P value*
Cohort 1a-14d-2m					
No of participants	**60**	**60**	**30**	**150**	—
Total	**1(2%)**	**2(3%)**	**0(0%)**	**3(2%)**	**0.80**
Hepatobiliary disorders					
Autoimmune hepatitis	1(2%)	0(0%)	0(0%)	1(1%)	1.00
Injury, poisoning and procedural complications					
Ankle fractures	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Neoplasms benign, malignant and unspecified (incl cysts and polyps)					
Teratoma	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Uterine leiomyoma	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Reproductive system and breast disorders					
Pelvic adhesions	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Gastrointestinal disorders					
Erosive gastritis	1(2%)	0(0%)	0(0%)	1(1%)	1.00
Cohort 1b-14d-8m					
No of participants	**60**	**60**	**30**	**150**	—
Total	**2(3%)**	**2(3%)**	**0(0%)**	**4(3%)**	**0.69**
Injury, poisoning and procedural complications					
Soft tissue injury	0(0%)	1(1%)	0(0%)	1(1%)	1.00
Hand fracture	1(2%)	0(0%)	0(0%)	1(1%)	1.00
Nail injury	1(2%)	0(0%)	0(0%)	1(1%)	1.00
Gastroesophageal reflux disease					
Hemorrhoid	1(2%)	0(0%)	0(0%)	1(1%)	1.00
Renal and urinary disorders					
Cystitis glandularis	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Cohort 2a-28d-2m					
No of participants	**60**	**60**	**30**	**150**	—
Total	**0(0%)**	**1(2%)**	**0(0%)**	**1(1%)**	**1.00**
Surgical and medical procedures					
Induced abortion	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Musculoskeletal and connective tissue disorders					
Herniated intervertebral disc	—	—	—	—	—
Cohort 2b-28d-8m					
No of participants	**60**	**60**	**30**	**150**	—
Total	**0(0%)**	**1(2%)**	**0(0%)**	**1(1%)**	**1.00**

Adverse events (MedDRA 23.0)	3 μg group	6 μg group	Placebo	Total	P value*
Surgical and medical procedures					
Induced abortion	0(0%)	1(2%)	0(0%)	1(1%)	1.00
Musculoskeletal and connective tissue disorders					
Herniated intervertebral disc	—	—	—	—	—

P value* was calculated by Fisher exact probability method and of the comparison of incidence rate among three groups. Events occurred from immunization to 28 days post dose 3.

Table S11. Serious adverse events reported in adults aged 60 years and older (cohort 3-28d-8m)

Adverse Events (MedDRA 23.0)	1.5 μg group (N=100)	3 μg group (N=101)	6 μg group (N=99)	Placebo group (N=49)	Total (N=349)	P value*
Total	10(10%)	5(5%)	7(7%)	2(4%)	24(7%)	0.49
Nervous system disorders						
Cerebral infarction	1(1%)	1(1%)	0(0%)	0(0%)	2(1%)	1.00
Hypoxic-Ischemic encephalopathy	1(1%)	0(0%)	1(1%)	0(0%)	2(1%)	0.67
Transient ischemic attack	0(0%)	1(1%)	0(0%)	0(0%)	1(0%)	1.00
Neoplasms benign, malignant and unspecified (incl cysts and polyps)						
Lung neoplasm	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71
Lung adenocarcinoma	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71
Liver cancer	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71
Kidney cyst	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71
Gastrointestinal disorders	1(1%)	1(1%)	1(1%)	1(2%)	4(1%)	0.84
Duodenal Ulcer	0(0%)	0(0%)	0(0%)	1(2%)	1(0%)	0.14
Gastrointestinal perforation	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71
Gastritis	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Pancreatitis	0(0%)	1(1%)	0(0%)	0(0%)	1(0%)	1.00
Respiratory, thoracic and mediastinal disorders						
Pulmonary inflammation	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Pulmonary emphysema	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71
Chronic obstructive pulmonary disease	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Cardiac disorders						
Unstable angina pectoris	2(2%)	0(0%)	0(0%)	0(0%)	2(1%)	0.42
Atrial fibrillation	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71
Musculoskeletal and connective tissue disorders						
Osteoarthritis	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Lumbar spinal stenosis	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Vascular and lymphatic diseases						
Hypertension	0(0%)	1(1%)	1(1%)	0(0%)	2(1%)	0.83
Hepatobiliary disease						
Acute cholecystitis	0(0%)	0(0%)	1(1%)	0(0%)	1(0%)	0.42
Infection and infectious diseases						
Bronchitis	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71

Adverse Events (MedDRA 23.0)	1.5 μg group (N=100)	3 μg group (N=101)	6 μg group (N=99)	Placebo group (N=49)	Total (N=349)	P value*
Injury, poisoning and operative complications						
Tibial fracture	1(1%)	0(0%)	0(0%)	0(0%)	1(0%)	0.71
Surgical and medical procedures						
Hip arthroplasty	0(0%)	1(1%)	0(0%)	0(0%)	1(0%)	1.00
Reproductive system and breast disease						
Benign prostatic hyperplasia	0(0%)	0(0%)	0(0%)	1(2%)	1(0%)	0.14

P value* was calculated by Fisher exact probability method and of the comparison of incidence rate among four groups. Events occurred from immunization to 28 days post dose 3.