Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Inclusion and exclusion criteria

Participants were considered eligible for the study if they met the following criteria:

- Age range: healthy people aged 18-59 years old;
- General good health as established by medical history and physical examination;
- Since December 2019, the participant has not gone to Hubei province, overseas, or to a village/community where there had been COVID-19 cases, has not contacted with confirmed or suspected cases, are not in the quarantine period, and are not from a village/community where there were confirmed or suspected cases.
- Women of childbearing age are not pregnant (negative urine pregnancy test), are not breastfeeding, do not have pregnancy plan within the three months after enrollment, and have already taken effective contraceptive measures two weeks before enrollment;
- Participants are able and willing to complete the whole research procedure in about 14 months;
- Participants have the ability to understand the research procedures, to sign the informed consent voluntarily after explanation, and can comply with the requirements of the clinical research program.

Those who met the inclusion criteria were further evaluated for the following exclusion criteria:

- Confirmed, suspected, or asymptomatic COVID-19 cases;
- Those with positive antibody tests of the COVID-19;
- History of SARS virus infection (identified through self-report or on-site inquiry);
- Those with fever (axillary temperature >37.0 °C), dry cough, fatigue, nasal obstruction, runny nose, sore throat, myalgia, diarrhea, shortness of breath, and dyspnea within 14 days before inoculation;
- Those with clinically abnormal parameters from blood biochemical, blood routine, and urine routine before inoculation (only for stage 1 clinical trial);
- Axillary temperature >37.0 °C before inoculation;
- Those who have experienced severe allergic reactions (such as acute anaphylaxis, urticaria, eczema, dyspnea, neurovascular edema, or abdominal pain), or those who are allergic to the known gradients of COVID-19 inactivated vaccine;
- Those with history or family history of convulsion, epilepsy, encephalopathy, or mental illness;
- Those with congenital malformation, developmental disorder, genetic defect, or severe malnutrition, etc.;
- Those with confirmed or suspected serious respiratory diseases, serious cardiovascular disease, severe liver or renal diseases, malignant tumors, uncontrolled hypertension (systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg), diabetes complications, malignant tumors, or various acute or chronic diseases (acute attack stage);
- Those diagnosed with congenital or acquired immunodeficiency, HIV infection, lymphoma, leukemia, or other autoimmune diseases;
- Those with a history of abnormal coagulation (such as lack of coagulation factors or coagulation diseases);
- Those receiving anti-TB treatment;
- Those receiving immune-enhancement or inhibitor treatment (p.o. or gtt.) over 14 days within 3 months (continuous oral or infusion for more than 14 days);
- Those receiving live-attenuated vaccines within one month before injectio or other vaccines within 14 days before inoculation;
- Those receiving blood products within 3 months before inoculation;
- Those receiving other study drugs within 6 months before inoculation;

— Those under other conditions not suitable for the clinical trial (evaluated by researchers).

After the first injection and before the subsequent second or third injection, the following participants were not allowed to be injected for the second or third injection or should be postponed for the injection:

— Women with positive urine pregnancy tests;
— Those with high fever (axillary temperature ≥39.0 °C) lasting for three days or severe allergic reaction after the previous injection;
— Serious adverse reactions related to the previous injection;
— If the investigators found that the participant did not meet the inclusion criteria or the participant met the exclusion criteria after the first dose, investigators should decide whether the participants could continue to participate in the study;
— Other reasons for exclusion evaluated by investigators.

Preparation of the inactivated SARS-CoV-2 vaccine

Two-steps of inactivation and multiple filtration and chromatography were used for the preparation of the vaccine. Briefly, the virus was inoculated and cultured on qualified Vero cells, and the harvested viral supernatants were inactivated with 1:4000 (v/v) β-propanolide for 48h at 2-8°C. After cell debris clarification and ultrafiltration, β-propanolide treatment were repeated once and the inactivation was validated by passing the treated samples onto Vero cells for 3 generations without cytopathic effects, then followed by purification using gel-chromatography, ion-exchange chromatography and filtration. The purified antigen was adsorbed on the Aluminum hydroxide (Alum) and subjected to the subsequent steps including filling, packaging and labeling. Applicable methods including dot-blotting, quantitative Real-Time PCR were conducted for the determination of residues of host Vero cell proteins, DNAs and additives in cell culture.

Detection of total specific IgG binding antibodies in serum samples with ELISA

The ELISA plates were coated with 1 μg/ml (100 μl/well) inactivated, purified whole-virus in Carbonate Buffer Solution (0.05 M, pH 9.6), at 4°C overnight, and then blocked with 200 μl blocking buffer containing 0.01 M Phosphate Buffered Saline with Tween (PBST)-1% bovine serum albumin (BSA) at 37°C for 1 hour. 100 μl 2-fold series of diluted serum samples were added to each well. After 1 hour of incubation at 37°C, plates were washed and incubated with 10 μl, 1:5000 diluted Horseradish Peroxidase (HRP)-conjugated antibodies against Human IgG (Boster Biological Technology, Ltd., Wuhan, China) for 1 hour at 37°C. The substrates of 1.25 mM 3', 3', 5, 5'-Tetramethyl benzidine and 6.52 mM Urea hydrogen peroxide were added (50 μl/ well) and incubated for 30 minutes, prior to addition of stop solution. Standard washing with PBST were conducted between each step. OD450 and OD630 values were measured, and positive were defined as higher than the cut-off value (0.15).

The specificity of the assay was tested by applying the method to 14 blood samples collected in 2017-2018, and none of the samples were tested positive (above the lower limit of detection), thus the specificity was considered as100%. In addition, none of the samples collected before the first dose in the phase 1 and 2 trials were tested positive, neither for the samples collected during the trial for those in the alum-only groups, which further indicated the high specificity of the assay. The recovery was tested by series of dilution (1×, 4×, and 16×) of a serum sample with a confirmed value of 1280 for the IgG test, and the samples were tested in triplicate

for each dilution, and the recovery ranged from 96.0%-105.3%. The inter-assay and intra-assay precision was tested by testing in triplicates of two samples with known high and low antibody titers in different assay runs or in the sample run. The coefficient variations were all below 11.0% (6.42%-10.21%).

Plaque Reduction Neutralization Test (PRNT)

All serum samples were heat-inactivated by incubation at 56°C for 30 min before use. The serum samples were diluted 20-fold first, and then 4-fold serial dilutions were prepared in maintenance medium. The virus suspension (0.25 ml, 600 plaque-forming units [PFU]/ml) was mixed with equal volume of serum at desirable dilution and incubated for 1 hour. The mixture was added to monolayer Vero E6 cells in a 12-well plate and incubated for 1 hour. Following removal of the mixture, 2 ml of maintenance medium containing 0.9% of methylcellulose (Sigma-Aldrich, Inc., St. Louis, MO, USA) were added to each well. The plates were incubated in a 5% CO_2-air incubator at 37°C for 3-4 days. Once plaques were developed in wells and observed by naked eye, then cells were fixed with 8% formaldehyde for 10 min at room temperature. Cells were stained with 0.5% crystal violet solution and the plaques were counted under the inversion microscope. The neutralizing titer was calculated as reciprocal of the highest serum dilution with 50% reduction of plaque forming. If the blank control results were not within the range of 30 ~ 300 PFU/well, the experiment will be performed repeated. Plaque reduction neutralizing antibody titer ($PRNT_{50}$, 95% CI, challenge viruses used: 30-300 PFU/well) was calculated as the “inhibitor vs normalized response (Variable slope)” model in the GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA).

The PRNT is the current laboratory standard for measuring neutralizing antibody. Standardization of the procedure using appropriate reference material, followed by each laboratory's own qualification and validation may result in better inter-laboratory comparisons of results. However, currently no reference material is available. Therefore, we have decided to measure all samples in triplicate on different days in the study to increase the accuracy and reliability of the results. But the serum samples collected before the first injection, 4 days and 14 days after the first injection in the phase 1 trial, and before the first injection in the phase 2 trial, were not repeated because all samples were below the lower limit of detection and there was no need to repeat the assays. The following principles were used to clean the data before analysis: 1) in general, the mean of the three repeated measures were taken as the $PRNT_{50}$ value of the sample; 2) if one of the three values fell out of the range (half to twice of the median titer), then the average of the two titers exhibiting closest agreement were used for further analysis; 3) if two of the three values fell out of the range (half to twice of the median titer), then the median titer were used for further analysis. The cut-off points were chosen based on results of the internal controls that were repeated every day. Because the values were obtained based on series of dilutions, a 2-fold difference was commonly seen and acceptable in this assay.

The specificity of the assay was tested in the similar way as the specific IgG binding antibody measurement, and was considered as100%. In addition, none of the samples collected before the first dose in the phase 1 and 2 trials were tested positive, neither for the samples collected during the trial for those in the alum-only groups, which further indicated the high specificity of the assay. The recovery was tested by series of dilution (1×, 10×, 100×, and 1000×) of a serum sample with a high neutralizing antibody titer, and the samples were tested in triplicate for each dilution, and the recovery ranged from 92.0%-95.0%. The inter-assay and intra-assay precision was tested by measuring five quality control samples of different titers in different days and also in triplicates within the same day. The geometric coefficient variations ranged from 19.8%-52.3%.

Lymphocyte subsets analysis

Lymphocyte subsets analysis were conducted to identify and determine the percentages and absolute counts of T, B, and natural killer (NK) cells as well as the $CD3^+$, $CD4^+$, and $CD8^+$ subpopulations of T cells in peripheral blood using fluorescent-activated cell sorting (FACS) method according to the manufacturer's manual of the Becton, Dickinson and Company's (BD) Multitest™ 6-color TBNK reagent. Briefly, 50 μl pre-mixed anticoagulant blood specimens were mixed with 20 μl BD Multitest™ 6-color TBNK reagent (Catalog No. 644611) in the BD Trucount™ absolute counting tube (Catalog No. 340334) and incubated for 20 min in the dark at room temperature, then treated with Beckman's OptiLyse C No-Wash Lysing Solution (Catalog No. A11895) for 10 min in the dark at room temperature. Cells were obtained by flow cytometry (BD FACS Canto II Cell Analyzer, Franklin Lakes, NJ, USA) and analyzed by BD FACSDiva™ software.

Cytokines detection analysis

Interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12P70, IL-17A, IL-17F, IL-21, interferon (IFN)-γ, tumor necrosis factor (TFN)-α, TFN-β cytokines tests were performed according to manufacturer's manual using the detection kit (Weimi Bio-Tech Co., Ltd., Guangzhou, China). In Brief, 25 μl serum samples were added to the 10 μl diluted micropellet mixture, then incubated with 10 μl diluted fluorescent labeled antibodies in the dark at room temperature for 2.5 h, after washed and centrifuged 200 g for 5 min with 1 ml Phosphate Buffered Saline (PBS), the supernatants were removed and replaced by 150 μl PBS. Two-fold series dilution of standard samples were also treated accordingly, and the negative controls were prepared using PBS instead of microspheres. Samples were objected to flow cytometry (BD FACS Canto II Cell Analyzer, Franklin Lakes, NJ, USA) and analyzed by BD FACSDiva™ software.

eTable 1. Grading Scales for Systemic and Local Adverse Events

Symptoms	Grade 1	Grade 2	Grade 3	Grade 4
Systemic adverse events				
Acute allergic reaction	Local urticaria (blister), no treatment required	Local urticaria, requiring treatment or mild angioedema, not requiring treatment	Extensive urticaria or angioedema requiring treatment or mild bronchospasm	Anaphylactic shock or life-threatening bronchospasm or laryngeal edema
Anorexia	Decreased appetite, but not reduced food intake	Decreased appetite and food intake, but no significant weight loss	Loss of appetite and weight	Interventions needed (e.g. tube feeding, parenteral nutrition)
Arthralgia	Mild pain, no impairment of function	Moderate pain; requires painkillers and/or pain impairs function but does not affect daily activities	Severe pain; need painkillers and/or pain affects daily activities	Disability pain
Constipation	Need stool softener and diet adjustment	Need defecation medicine	Intractable constipation needs manual dredging or enema	Toxic megacolon or intestinal obstruction
Coughing	Transient, no treatment required	Persistent cough, effective treatment	Paroxysmal cough, uncontrollable by treatment	Emergency or hospitalization
Diarrhea	Slight or transient, 3-4 times/day, abnormal fecal characteristics, or slight diarrhea lasting less than 1 week	Moderate or persistent, 5-7 times/day, abnormal fecal characteristics, or diarrhea > 1 week	>7 times/day, abnormal fecal characteristics, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, need intravenous infusion > 2L	Hypotension shock, requiring hospitalization
Dysphagia	Mild discomfort when swallowing	Restricted diet	Limited diet and conversation; unable to eat solid food	Unable to eat liquid food; requiring intravenous nutrition
Dyspnea	Dyspnea during exercise	Dyspnea due to normal activities	Dyspnea at rest	Dyspnea, requiring oxygen therapy, hospitalization or assisted respiration
Fatigue	No impact on daily	Affect normal daily	Seriously affect	Emergency or

Symptoms	Grade 1	Grade 2	Grade 3	Grade 4
	activities	activities	daily activities, unable to work	hospitalization
Fever (axillary temperature, ℃)	37.3 ~ <38.0	38.0 ~ <38.5	38.5 ~ <39.5	≥39.5, lasting for more than 3 days
Headache	No impact on daily activities, no need for treatment	Temporary, slight impact on daily activities, may need treatment or intervention	Seriously affecting daily activities, requiring treatment or intervention	Intractable, requiring emergency treatment or hospitalization
Mucocutaneous abnormalities	Erythema/pruritus/color change	Diffuse rash/macula/dryness/desquamation	Blister/exudation/desquamation/ulcer	Exfoliative dermatitis involving mucosa, erythema multiforme, or Stevens Johnson syndrome
Myalgia (non-injection sites)	No impact on daily activities	Slightly affect daily activities	Severe muscle pain, serious impact on daily activities	Emergency or hospitalization
Nausea	Transient (<24 hours) or intermittent and food intake is basically normal	Continuous nausea leads to reduced food intake (24-48 hours)	Persistent nausea results in almost no food intake (>48 hours) or need intravenous rehydration	Life threatening (such as hypotension shock)
Pruritus (non-injection site)	Mild, not or slightly affect daily life	Pruritus affects daily life	Pruritus leads to carry out daily life	NA
Vomiting	1-2 times/24 hours without affecting the activity	3-5 times/24 hours or limited activity	More than 6 times in 24 hours or need intravenous rehydration	Need to be hospitalized or other ways of nutrition due to hypotension shock
Systemic adverse events				
Pain	No or slight impact on physical activity	Affect physical activity	Affect daily life	Loss of basic self-care ability or hospitalization
Swelling	Diameter of 2.5 ~ <5 cm or area of 6.25 ~ <25 cm^2, not 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	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis

Symptoms	Grade 1	Grade 2	Grade 3	Grade 4
			serious impact on daily life	
Redness	Diameter of 2.5 ~ <5 cm or area of 6.25 ~ <25 cm^2, not 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
Itching	Pruritus on the inoculation site, relieved by itself or within 48 hours after treatment	Pruritus on inoculation site did not relieve within 48 hours after treatment	Affect daily life	NA

Abbreviation: NA, not available.

eTable 2. Total Adverse Events after Each Dose in the Phase 1 and 2 Trials[a]

Symptom	Procedure	Group	N	Dose		
				1	2	3
0-7 days						
Total adverse reactions	0, 28 & 56 day	Low dose	24	1 (4.2)	3 (12.5)	2 (8.3)
		Medium dose	24	2 (8.3)	1 (4.2)	1 (4.2)
		High dose	24	3 (12.5)	4 (16.7)	1 (4.2)
		Alum-only	24	2 (8.3)	0	1 (4.2)
	0 & 14 day	Medium dose	84	4 (4.8)	1 (1.2)	NA
		Alum-only	28	4 (14.3)	0	NA
	0 & 21 day	Medium dose	84	9 (10.7)	9 (10.7)	NA
		Alum-only	28	5 (17.9)	3 (10.7)	NA
Systemic reactions	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	1 (4.2)	1 (4.2)	1 (4.2)
		High dose	24	1 (4.2)	0	0
		Alum-only	24	1 (4.2)	0	0
	0 & 14 day	Medium dose	84	4 (4.8)	0	NA
		Alum-only	28	2 (7.1)	0	NA
	0 & 21 day	Medium dose	84	3 (3.6)	1 (1.2)	NA
		Alum-only	28	2 (7.1)	0	NA
Coughing	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	0	0
		High dose	24	0	0	0
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	1 (1.2)	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA
Diarrhea	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	0	0
		High dose	24	0	0	0
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	1 (1.2)	0	NA
		Alum-only	28	0	0	NA
Fatigue	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	0	1 (4.2)
		High dose	24	0	0	0
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	1 (1.2)	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	0	0	NA

Symptom	Procedure	Group	N	Dose		
				1	2	3
		Alum-only	28	0	0	NA
Fever	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	1 (4.2)	0	0
		High dose	24	1 (4.2)	0	0
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	4 (4.8)	0	NA
		Alum-only	28	1 (3.6)	0	NA
	0 & 21 day	Medium dose	84	1 (1.2)	1 (1.2)	NA
		Alum-only	28	1 (3.6)	0	NA
Headache	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	0	0
		High dose	24	0	0	0
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	1 (1.2)	0	NA
		Alum-only	28	1 (3.6)	0	NA
	0 & 21 day	Medium dose	84	0	0	NA
		Alum-only	28	1 (3.6)	0	NA
Nausea and vomiting	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	1 (4.2)	0
		High dose	24	0	0	0
		Alum-only	24	1 (4.2)	0	0
	0 & 14 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	1 (1.2)	0	NA
		Alum-only	28	1 (3.6)	0	NA
Pruritus (non-inoculated site)	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	0	0
		High dose	24	0	0	0
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	0	0	NA
		Alum-only	28	1 (3.6)	0	NA
Local reactions	0, 28 & 56 day	Low dose	24	1 (4.2)	3 (12.5)	2 (8.3)
		Medium dose	24	1 (4.2)	0	0
		High dose	24	2 (8.3)	4 (16.7)	1 (4.2)
		Alum-only	24	1 (4.2)	0	1 (4.2)
	0 & 14 day	Medium dose	84	1 (1.2)	1 (1.2)	NA
		Alum-only	28	3 (10.7)	0	NA
	0 & 21 day	Medium dose	84	7 (8.3)	8 (9.5)	NA

Symptom	Procedure	Group	N	Dose		
				1	2	3
		Alum-only	28	5 (17.9)	3 (10.7)	NA
Itching	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	0	0
		High dose	24	0	0	0
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	1 (1.2)	0	NA
		Alum-only	28	1 (3.6)	0	NA
Pain	0, 28 & 56 day	Low dose	24	1 (4.2)	3 (12.5)	2 (8.3)
		Medium dose	24	1 (4.2)	0	0
		High dose	24	2 (8.3)	4 (16.7)	0
		Alum-only	24	1 (4.2)	0	1 (4.2)
	0 & 14 day	Medium dose	84	1 (1.2)	1 (1.2)	NA
		Alum-only	28	3 (10.7)	0	NA
	0 & 21 day	Medium dose	84	5 (6.0)	8 (9.5)	NA
		Alum-only	28	4 (14.3)	3 (10.7)	NA
Redness	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	0	0
		High dose	24	0	0	1 (4.2)
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	0	0	NA
		Alum-only	28	1 (3.6)	0	NA
Swelling	0, 28 & 56 day	Low dose	24	0	0	1 (4.2)
		Medium dose	24	0	0	0
		High dose	24	0	0	1 (4.2)
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	1 (1.2)	0	NA
		Alum-only	28	1 (3.6)	0	NA
Other reactions	0, 28 & 56 day	Low dose	24	0	0	0
		Medium dose	24	0	0	0
		High dose	24	0	0	0
		Alum-only	24	0	0	0
	0 & 14 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA
	0 & 21 day	Medium dose	84	0	0	NA
		Alum-only	28	0	0	NA

Symptom	Procedure	Group	N	Dose		
				1	2	3
0-28 days						
Total adverse reactions	0, 28 & 56 day	Low dose	24	1 (4.2)	3 (12.5)	2 (8.3)
		Medium dose	24	2 (8.3)	1 (4.2)	1 (4.2)
		High dose	24	3 (12.5)	4 (16.7)	1 (4.2)
		Alum-only	24	2 (8.3)	0	1 (4.2)
	0 & 14 day	Medium dose	84	4 (4.8)	1 (1.2)	NA
		Alum-only	28	4 (14.3)	0	NA
	0 & 21 day	Medium dose	84	9 (10.7)	9 (10.7)	NA
		Alum-only	28	5 (17.9)	3 (10.7)	NA

Abbreviation: NA, not available.

[a] Data are shown as No. of participants with event (%).

eTable 3. Total Adverse Events 28 Days after Three Doses in the Phase 1 Trial and Two Doses in the Phase 2 Trial[a]

Adverse Events	Phase 1 Clinical Trial				Phase 2 Clinical Trial			
	0, 28 & 56 Day Group				0 & 14 Day Group		0 & 21 Day Group	
	Low Dose (N = 24)	Medium Dose (N = 24)	High Dose (N = 24)	Alum-only (N = 24)	Medium Dose (N = 84)	Alum-only (N = 28)	Medium Dose (N = 84)	Alum-only (N = 28)
Total adverse events	6 (25.0)	4 (16.7)	10 (41.7)	4 (16.7)	7 (8.3)	4 (14.3)	21 (25.0)	7 (25.0)
Systemic events	0	3 (12.5)	2 (8.3)	2 (8.3)	5 (6.0)	2 (7.1)	5 (6.0)	2 (7.1)
Coughing	0	0	0	0	2 (2.4)	0	0	0
Diarrhea	0	0	0	0	0	0	1 (1.2)	0
Fatigue	0	1 (4.2)	0	1 (4.2)	1 (1.2)	0	0	0
Fever	0	1 (4.2)	1 (4.2)	0	4 (4.8)	1 (3.6)	3 (3.6)	1 (3.6)
Headache	0	0	0	0	1 (1.2)	1 (3.6)	0	1 (3.6)
Nausea and vomiting	0	1 (4.2)	1 (4.2)	1 (4.2)	0	0	1 (1.2)	1 (3.6)
Anorexia	0	0	1 (4.2)	0	0	0	0	0
Pruritus (non-injection site)	0	0	0	0	0	0	0	1 (3.6)
Local events	5 (20.8)	1 (4.2)	6 (25.0)	2 (8.3)	2 (2.4)	3 (10.7)	13 (15.5)	4 (14.3)
Itching	0	0	0	0	0	0	1 (1.2)	1 (3.6)
Pain	5 (20.8)	1 (4.2)	6 (25.0)	2 (8.3)	2 (2.4)	3 (10.7)	12 (14.3)	4 (14.3)
Redness	0	0	1 (4.2)	0	0	0	0	1 (3.6)
Swelling	1 (4.2)	0	1 (4.2)	0	0	0	1 (1.2)	1 (3.6)
Other events	3 (12.5)	1 (4.2)	5 (20.8)	0	3 (3.6)	1 (3.6)	5 (6.0)	2 (7.1)
Grade III adverse events	0	0	2 (8.3)[b]	0	0	0	2 (2.4)[c]	0
Total adverse events after each dose								
First dose	2 (8.3)	2 (8.3)	4 (16.7)	2 (8.3)	6 (7.1)	4 (14.3)	13 (15.5)	6 (21.4)
Second dose	4 (16.7)	1 (4.2)	8 (33.3)	1 (4.2)	1 (1.2)	1 (3.6)	11 (13.1)	4 (14.3)
Third dose	2 (8.3)	1 (4.2)	1 (4.2)	1 (4.2)	-	-	-	-

[a]The low, medium and high dose represents 2.5, 5 and 10 μg/dose. Data are shown as No. of participants with event (%). Participants could have more than one adverse event.

[b]There were two grade 3 adverse events occurred in this group: one was swelling and pain of left knee joint and subcutaneous hematoma after falling on the ground, this occurred 25 days after the second injection; the other one was abdominal pain and subsequent diagnosis of acute appendicitis, this occurred 27 days after the first injection. Both events were judged not related to the vaccination.

[c]There were two grade 3 adverse events occurred in this group: one was fever of 39.0 °C on day 11 after the second injection. The participant took some medications and was relieved one day later. The other adverse event was multiple skin contusion in maxillofacial region 7 days after the second injection. The symptoms included skin laceration from left eyebrow arch to hair source, multiple skin contusion at nasal back, slight swelling of lip, laceration of upper lip, slight swelling of external nose, and proliferation of lymphatic follicles in posterior pharyngeal wall. The participant was hospitalized for treatment and the symptoms were relieved after treatment for a week. Both events were judged not related to the vaccination.

eTable 4. Abnormal Laboratory Parameters after three Vaccinations in the Phase 1 Clinical Trial[a]

Laboratory parameters[b]	Low Dose (N = 24)	Medium Dose (N = 24)	High Dose (N = 24)	Alum-only (N = 24)
Total lymphocyte count	2 (8.3)	1 (4.2)	1 (4.2)	2 (8.4)
Hemoglobin	0	0	0	1 (4.2)
Total bilirubin	1 (4.2)	1 (4.2)	3 (12.5)	0
Alanine aminotransferase	1 (4.2)	0	0	1 (4.2)
Aspartate aminotransferase	0	0	0	1 (4.2)
Creatinine	0	1 (4.2)	0	
Blood urea nitrogen	0	0	1 (4.2)	0
Fasting blood glucose	1 (4.2)	2 (8.3)	1 (4.2)	0
Urinary protein	2 (8.3)	0	0	1 (4.2)
Urinary sugar	1 (4.2)	1 (4.2)	0	0
Urinary red blood cell	2 (8.3)	1 (4.2)	4 (16.7)	2 (8.3)

[a] The low, medium and high dose represents 2.5, 5 and 10 μg/dose. The laboratory parameters were tested before each dose and 4 days after each dose. All participants were in the normal range of the parameters before the first dose, and the numbers shown in the table are number of participants (not number of times) with abnormal values after the first dose.

[b] The abnormal value of each parameter was defined as follows: $\leq 1\times10^9$ /L for total lymphocyte count, <11.0 g/dL (for men) or <10.5 g/dL (for women) for hemoglobin, ≥1.1×upper limit of normal value for total bilirubin and creatinine, ≥1.25×upper limit of normal value for alanine aminotransferase, aspartate aminotransferase, and blood urea nitrogen, ≥6.11 mmol/L or <3.55 mmol/L for fasting blood glucose, 1+ or more for urinary protein, trace or more for urinary sugar, and ≥6/ high power field for urinary red blood cell.

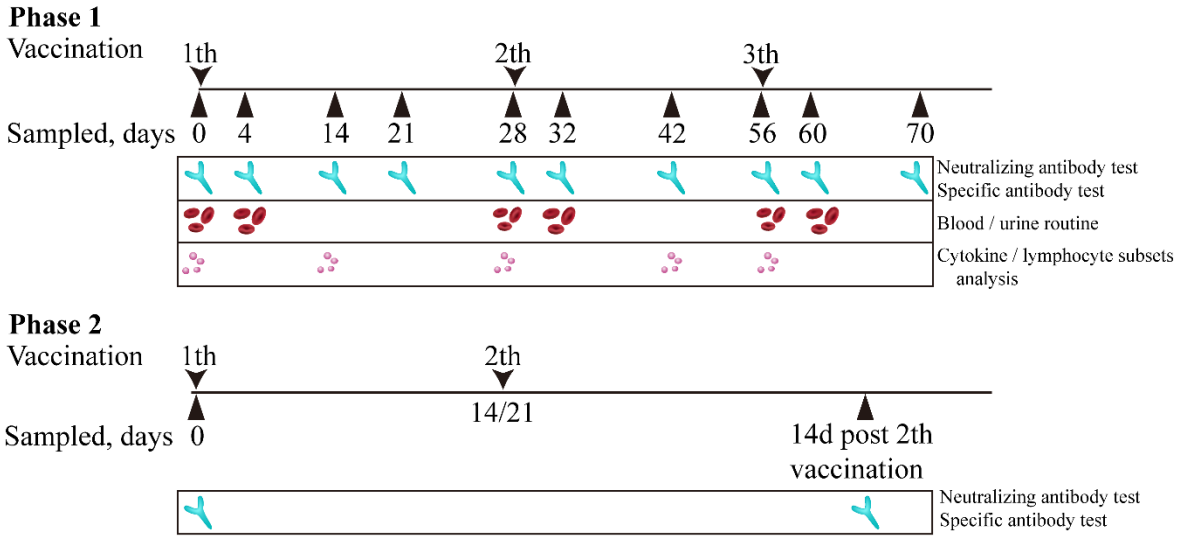
eTable 5. Geometric Mean Titer (95% Confidence Interval) of Antibodies in Different Time Points in the Two Trials.[a]

Variable	Phase 1 Clinical Trial				Phase 2 Clinical Trial			
	0, 28 & 56 Day Group				0 & 14 Day Group		0 & 21 Day Group	
	Low Dose (N = 24)	Medium Dose (N = 24)	High Dose (N = 24)	Alum-only (N = 24)	Medium Dose (N = 42)	Alum-only (N = 14)	Medium Dose (N = 42)	Alum-only (N = 14)
Neutralizing Antibodies to Live SARS-CoV-2								
Before first dose	5 (5-5)	5 (5-5)	5 (5-5)	5 (5-5)	5 (5-5)	5 (5-5)	5 (5-5)	5 (5-5)
4 days after first dose	5 (5-5)	5 (5-5)	5 (5-5)	5 (5-5)	-	-	-	-
14 days after first dose	5 (5-5)	6 (5-7)	6 (4-8)	5 (5-5)	-	-	-	-
21 days after first dose	5 (5-5)	7 (5-10)	6 (4-8)	5 (5-5)	-	-	-	-
Before second dose	5 (5-5)	12 (7-22)	8 (5-12)	5 (5-5)	-	-	-	-
4 days after second dose	20 (11-37)	13 (7-23)	7 (5-11)	5 (5-5)	-	-	-	-
14 days after second dose	89 (49-164)	129 (68-243)	101 (59-172)	5 (5-5)	114 (89-146)	5 (5-5)	252 (179-356)	5 (5-5)
Before third dose	94 (56-159)	125 (83-188)	93 (54-161)	5 (5-5)	-	-	-	-
4 days after third dose	97 (56-166)	137 (91-206)	118 (70-198)	5 (5-5)	-	-	-	-
14 days after third dose	316 (218-457)	206 (123-343)	297 (208-424)	5 (5-5)	-	-	-	-
Specific Antibody Responses to SARS-CoV-2								
Before first dose	10 (10-10)	10 (10-10)	10 (10-10)	10 (10-10)	10 (10-10)	10 (10-10)	10 (10-10)	10 (10-10)
4 days after first dose	10 (10-10)	10 (10-10)	10 (10-10)	10 (10-10)	-	-	-	-
14 days after first dose	14 (11-17)	13 (11-16)	14 (11-18)	10 (10-10)	-	-	-	-
21 days after first dose	19 (16-24)	17 (14-22)	18 (14-23)	10 (10-10)	-	-	-	-
Before second dose	19 (15-23)	15 (11-19)	21 (16-26)	10 (10-10)	-	-	-	-
4 days after second dose	19 (15-24)	19 (14-26)	27 (21-34)	10 (10-10)	-	-	-	-
14 days after second dose	160 (93-276)	151 (106-215)	254 (159-406)	10 (10-10)	74 (56-97)	10 (10-10)	215 (157-296)	10 (10-10)
Before third dose	160 (105-244)	190 (142-254)	285 (196-414)	10 (10-10)	-	-	-	-
4 days after third dose	139 (96-200)	320 (237-432)	185 (143-239)	10 (10-10)	-	-	-	-

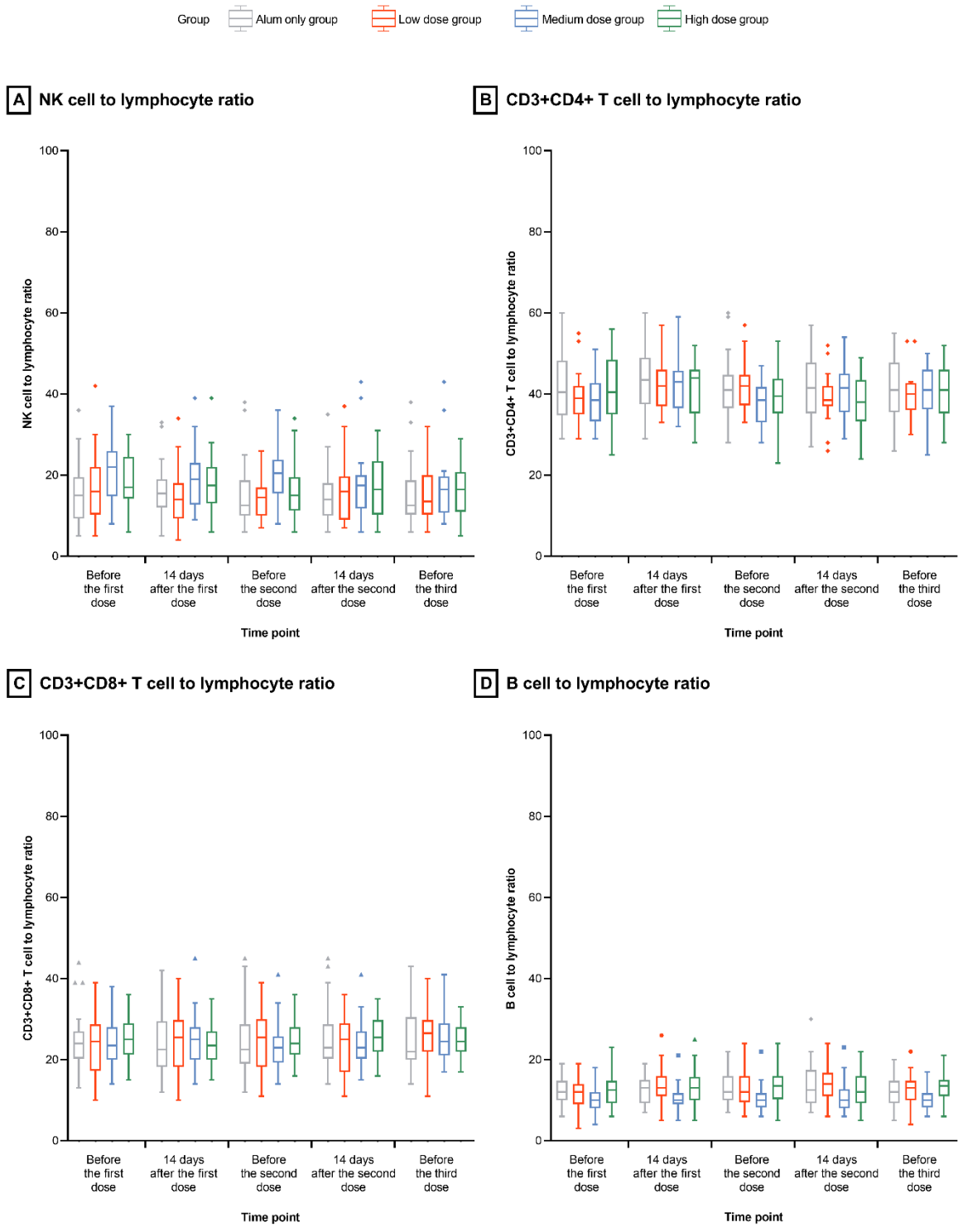
14 days after third dose	415 (288-597)	349 (258-472)	311 (229-422)	10 (10-10)	-	-	-	-

[a] Immunogenicity population is defined as randomized participants who received at least one dose injection with non-missing immunogenicity data before or after injections. All participants in the phase 1 trial and the first half of the participants in the phase 2 trial were scheduled for the hormoral immunogenicity measurement, and there were no missing data. The low, medium and high dose represents 2.5, 5 and 10 μg/dose. The baseline values were imputed by the lower limit of detection of the assays, which was 5 for the neutralizing antibody measurement and 10 for the specific IgG binding antibody measurement.

eFigure 1. Schedule of Biospecimen Collection and Related Tests

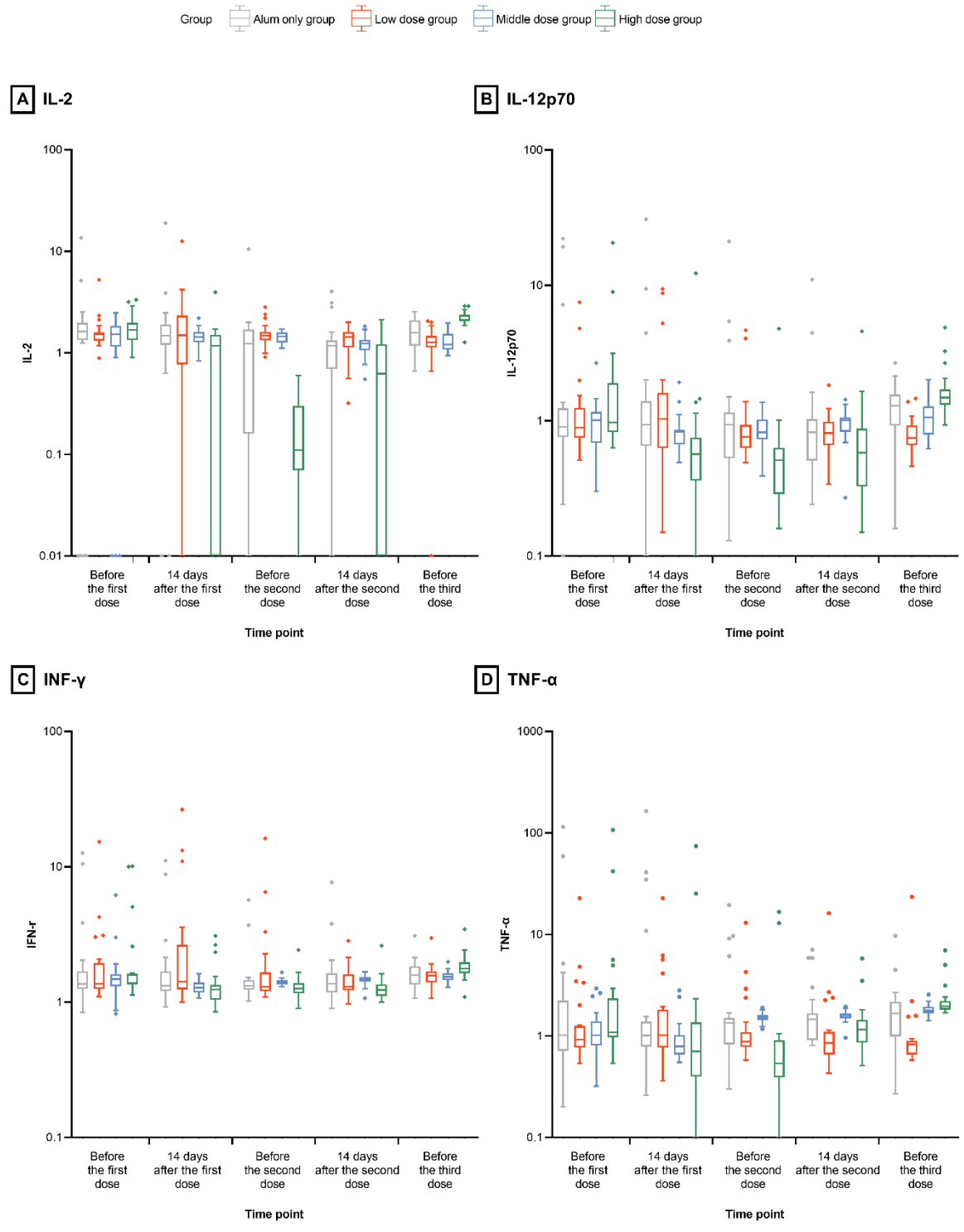


eFigure 2. Lymphocyte Subset Distribution Analysis in the Phase 1 Trial



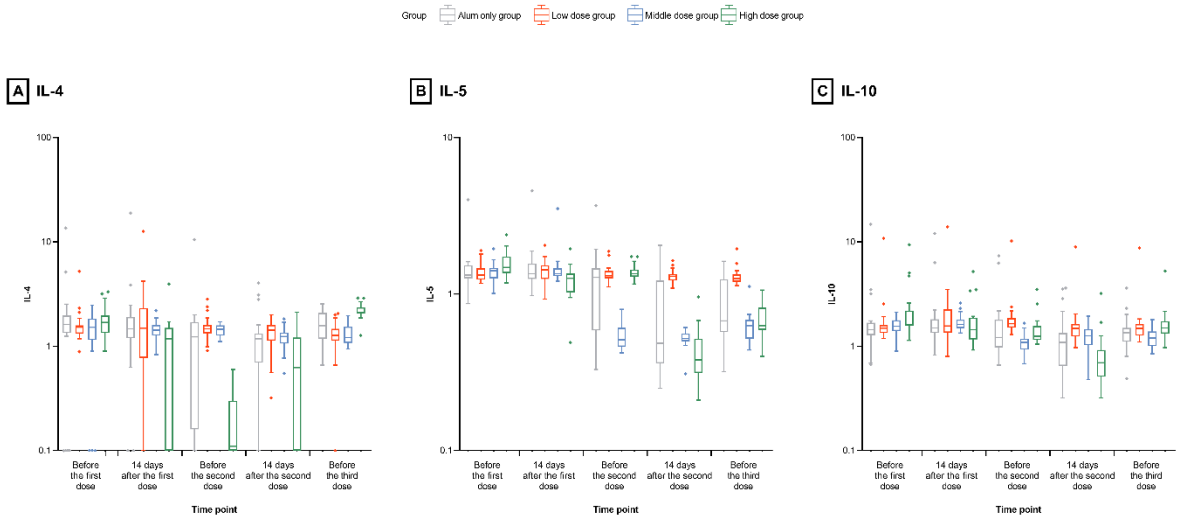
The boxes and horizontal bars denote interquartile range (IQR) and median value. The whisker's upper endpoint equaled to 75th percentile + 1.5×IQR or the maximum observation whichever is lower, and the whisker's lower endpoint equaled to 25th percentile - 1.5×IQR or the minimum observation whichever is higher.

eFigure 3. Changes in T Helper 1 Cell Related Cytokines in the Phase 1 Trial



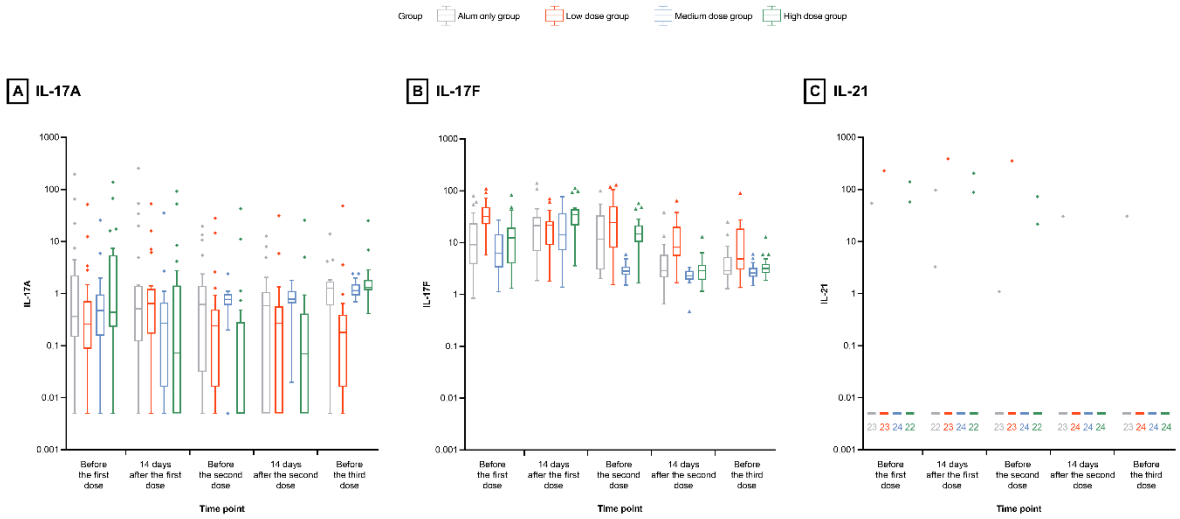
The boxes and horizontal bars denote interquartile range (IQR) and median value. The whisker's upper endpoint equaled to 75th percentile + 1.5×IQR or the maximum observation whichever is lower, and the whisker's lower endpoint equaled to 25th percentile - 1.5×IQR or the minimum observation whichever is higher. The unit for all the biomarkers is pg/mL.

eFigure 4. Changes in T Helper 2 Cell Related Cytokines in the Phase 1 Trial



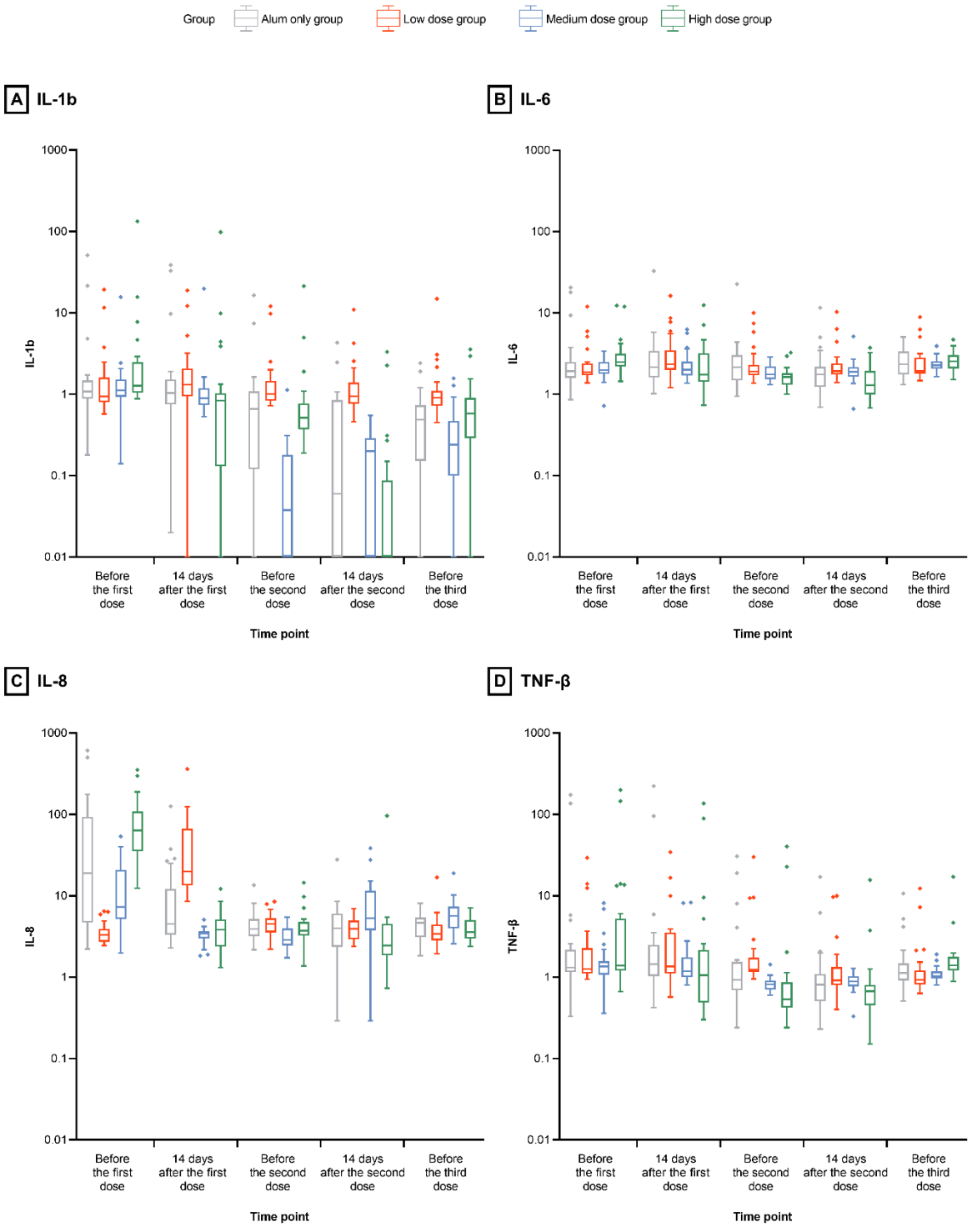
The boxes and horizontal bars denote interquartile range (IQR) and median value. The whisker's upper endpoint equaled to 75th percentile + 1.5×IQR or the maximum observation whichever is lower, and the whisker's lower endpoint equaled to 25th percentile - 1.5×IQR or the minimum observation whichever is higher. The unit for all the biomarkers is pg/mL.

eFigure 5. Changes in T Helper 17 Cell Related Cytokines in the Phase 1 Trial



The boxes and horizontal bars denote interquartile range (IQR) and median value. The whisker's upper endpoint equaled to 75th percentile + 1.5×IQR or the maximum observation whichever is lower, and the whisker's lower endpoint equaled to 25th percentile - 1.5×IQR or the minimum observation whichever is higher. The unit for all the biomarkers is pg/mL.

eFigure 6. Changes in Other Cytokines in the Phase 1 Trial



The boxes and horizontal bars denote interquartile range (IQR) and median value. The whisker's upper endpoint equaled to 75th percentile + 1.5×IQR or the maximum observation whichever is lower, and the whisker's lower endpoint equaled to 25th percentile - 1.5×IQR or the minimum observation whichever is higher. The unit for all the biomarkers is pg/mL.