Subjects inclusion/exclusion criteria

Inclusion criteria

1. The signed and dated informed consent form for the subject's participation in the study is available.
2. The subjects are healthy males and females aged 18-50 and over.
3. A subject is able to keep records in the Self-Observation Diary independently and to undergo the medical monitoring during the follow-ups stipulated in the study and does this on their own volition.
4. Females voluntarily use the methods of reliable contraception throughout the entire period of their participation in the study.
5. Tests for IgM and IgG antibodies to SARS-CoV-2 are negative.
6. There is no history of COVID-19 coronavirus infection.
7. Over the past 14 days, there was no close contact with individuals suspected of being infected with SARS-CoV-2, or individuals with a laboratory-confirmed diagnosis of coronavirus infection COVID-19.
8. Tests for human immunodeficiency virus (HIV), hepatitis B and hepatitis C are negative.

Exclusion criteria

1. There are known allergic reactions in history, intolerance to medication, including hypersensitivity to any of the components of the investigational product, beta-lactam antibiotics and aminoglycosides, as well as a history of serious adverse events after vaccination (such as allergic reactions, respiratory failure, angioedema, abdominal pain).
2. The acute condition with fever (body temperature ≥37.1 °C) is found at the screening/randomization stage.
3. The subject has a history of chronic alcohol and/or drug abuse.
4. Laboratory and/or instrumental examination at the screening stage shows clinically significant deviations from normal values.
5. Women have a positive urine pregnancy test.
6. The subject has received concurrent treatment with immunosuppressive agents, including corticosteroids (2 weeks), 4 weeks prior to the investigational product administration.
7. Medical history, physical examination, or clinical laboratory tests reveal conditions that may affect the study result, according to the investigator, including acute or chronic clinically significant disorders of the lungs, cardiovascular system, gastrointestinal tract, liver, or blood system, as well as skin, endocrine, neurological and psychiatric disorders or impaired renal function (asthma, diabetes, thyroid disease, arrhythmia, myocardial infarction, severe hypertension not controlled by medication, etc.).
8. There are platelet disorders or other blood-clotting disorders, which may cause contraindications to intramuscular administration.
9. The subject has a history of leukemia or neoplasm.
10. The subject has an autoimmune disease.
11. The subject has received antiviral medication, immunoglobulins, or blood transfusions or any other investigational product within 4 weeks before the studied investigational product administration;
12. The subject has received anti-inflammatory medicines 2 days before the investigational product administration;
13. The subject has participated in any other clinical trial within the last 3 months.
14. The subject is suspected not to comply with the study requirements or has an evident physical or mental disability that may affect the completion of the study.
15. The subject refuses to participate in the study voluntarily.
16. The subject is vulnerable.

Supplemental methods

i. Microneutralisation assay (MNA)

A 50% tissue culture infective dose ($TCID_{50}$)-based microneutralisation assay was used to detect and titrate neutralising antibodies. Serum samples were preheated at 56°C for 30 min to avoid complement-linked reduction of the viral activity. Serial two-fold dilutions of each serum specimen in Hank's solution, starting from 1:2, were prepared in 100 µl volume. Each dilution was mixed with an equal volume of live SARS-CoV-2 virus (100 $TCID_{50}$ doses diluted in DMEM (Sigma) supplemented with 2% FBS) and incubated at 37°C for 60 min. The mix was then transferred to 96-well microplates with monolayer Vero cells in quadruplicate. The plates were incubated in a 5% CO_2 atmosphere at 37°C and read microscopically 5 days later. Neutralisation was recorded if 50% or more of the inoculated wells showed no visible plaques or cytopathic effect (CPE). The serum neutralising titre was expressed as the highest serum dilution that exhibited neutralising activity. All experiments were performed in a BSL3 laboratory.

ii. ELISA

Commercial kit SARS-CoV-2-IgG-EIA-BEST (#D-5501, Vector-Best, Russia) and CAMOMILE –SARS-CoV-2 G/M (Diamed Asia Test, Kazakhstan) were used according to the manufacturer's instructions to detect IgG and IgM antibodies, specific to the recombinant Spike (S) SARS-CoV-2 protein. Briefly, serial 1:2 dilutions of serum samples were prepared, beginning from 1:100, and added to the plate coated with recombinant SARS-CoV-2 antigen. After 30 min incubation at 37°C at 700 rpm, plates were washed 5 times. Secondary antibody conjugate was then added to the wells, and incubation was performed at 37°C and 700 rpm for another 30 minutes. After the incubation, the plates were washed 5 times and TMB substrate was added to wells. Colorimetric reaction was allowed to develop at 18-25°C for 25 minutes and was then stopped by adding the Stop Solution. Optical density (OD) was measured at 450 nm. A threshold was set as mean OD in negative template control wells plus 0·2. Wells with OD ≥ 1·1 times higher than the threshold were recorded as positive. The antibody titre was expressed as the highest positive serum dilution.

iii. Whole-blood cytokine release analyses

For cytokine production measurement, 2 ml of fresh blood was mixed with 4 ml of DMEM, containing heparin (2·5 U/ml), gentamicin (100 µg/ml), and L-glutamine (0·6 mg/ml). To measure the antigen-induced cytokine production, 10 µl of purified Nucleocapsid and Spike proteins of SARS-CoV-2/human/KAZ/KZ Almaty/2020 virus (5 mg/ml) were added to 2 ml of diluted blood. No stimulators were added to the control samples. The tubes were incubated at 37°C for 24 h. After the incubation, samples were centrifuged at 3000 g for 10 min, and the supernatants were transferred to the new tubes, aliquoted and stored at -70°C until usage. The cytokine response (IFN-γ, TNF-α, IFN-α, IL-6, IL-4) was measured using ELISA kits (Cat. No A-8752, A-8756, A-8758, A-8768, A-8754, Vector-Best CJSC, Novosibirsk, Russia) following the manufacturer's instructions.

iv. Total serum IgE

Commercial kit ImmunoFA-IgE (Immunotekh, NVO, Russia) was used according to the manufacturer's instructions to measure total IgE antibodies in serum by direct ELISA. Briefly, control, calibrator and test serum samples were added in duplicates to the pre-coated plate (0·02 ml/well). The HRP-labelled anti-IgE conjugate was added right after (0·1 ml/well) and mixed. After 1 h incubation at 37°C, plates were washed and TMB substrate was added to wells (0.1 ml/well). Colorimetric reaction was allowed to develop at 37°C for 10-15 minutes in the dark and then was stopped by adding the Stop Solution (0·1 ml/well). Optical density was measured at 450 nm. IgE concentrations were calculated from the piecewise-linear calibration curve and expressed in IU/ml.

Supplemental figures

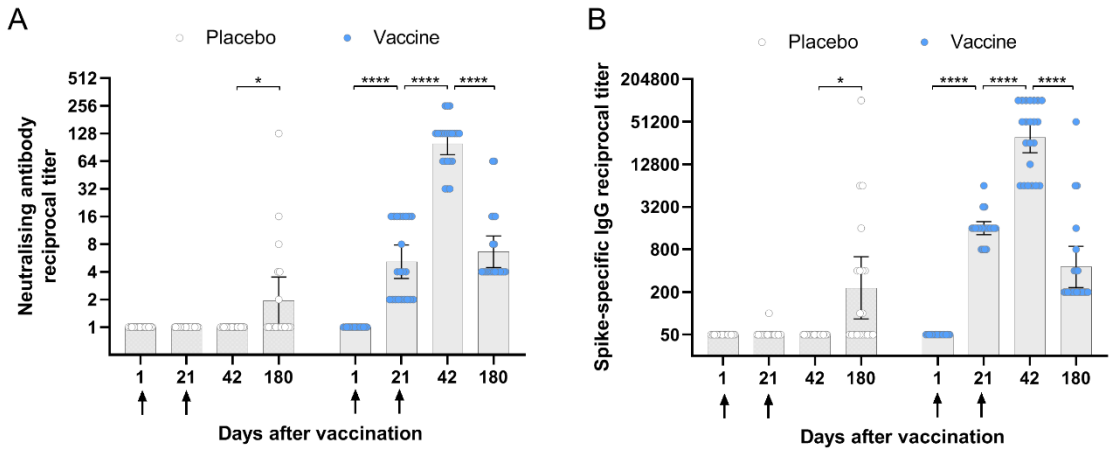

*Figure **S1***: **Humoral immune response in phase 1**
Neutralising and Spike-specific IgG antibody titres detected by MNA (A) and ELISA (B). Individual values for each subject are shown as circles. Grey bars with errors indicate GMTs with 95% CI.
Groups were compared by two-way RM-ANOVA (applied to the $\log_2$ values) followed by Bonferroni's multiple comparison test; *p<0·05 and **** p <0·0001 indicate statistically significant differences.

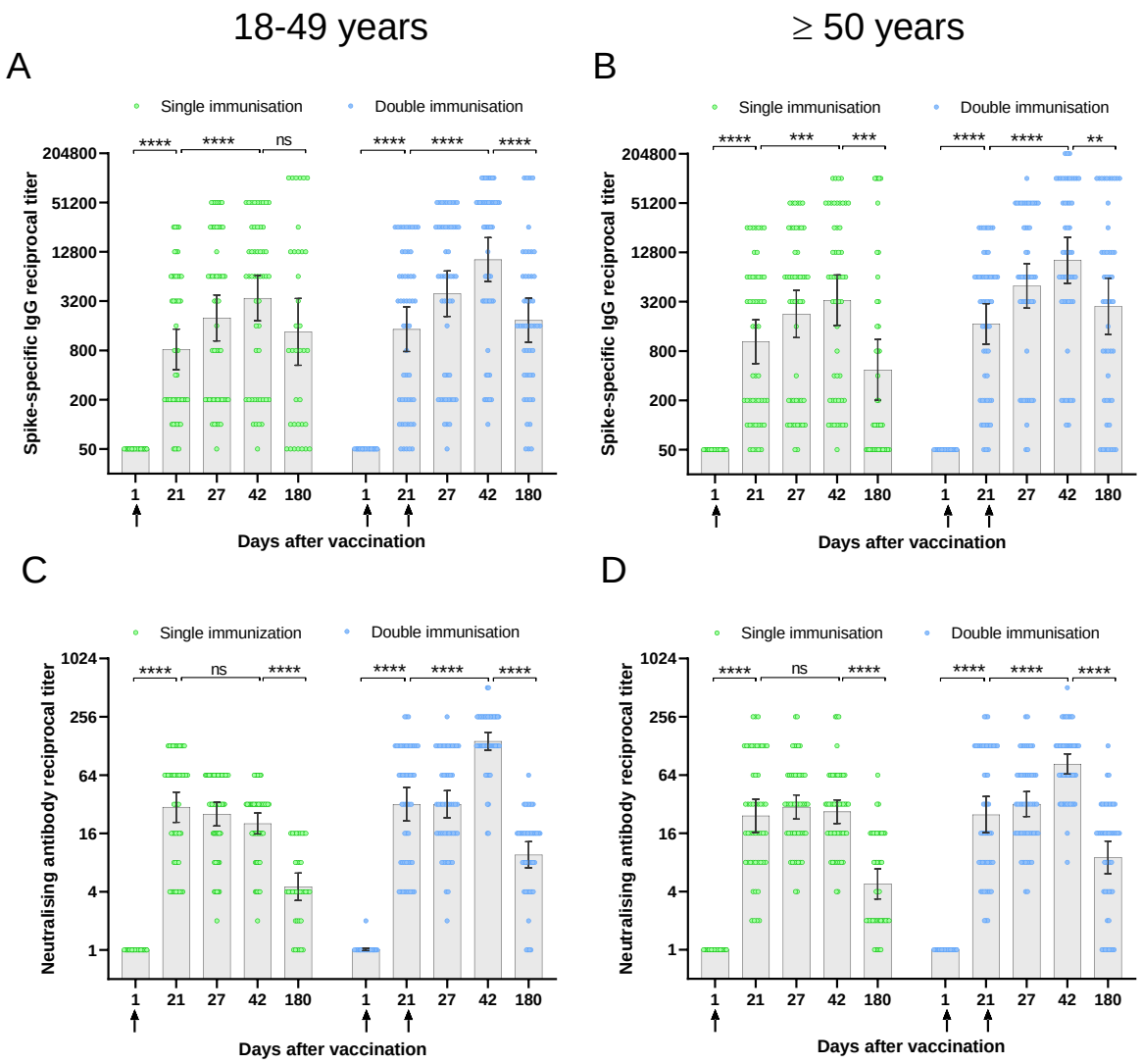

Figure ***S2*****: Humoral immune response in phase 2**
Serum antibody titres measured on day 1 (before vaccination), on days 21 and 27 (after the first vaccination), and on day 42 and 180 (after the second vaccination) are shown. (A, B) ELISA data; (C, D) MNA data. Individual values for each subject are shown as circles. Grey bars with errors indicate GMTs with 95% CI. GMT values were compared in post-hoc analyses by two-way RM-ANOVA (applied to the log2 values) followed by Bonferroni's multiple comparison test; ** p<0·01, **** p <0·0001, indicate statistically significant differences, ns - not significant.

Supplemental tables

	Vaccine (n=22)	Placebo (n=22)
Sex		
Female	5 (22·7%)	10 (45·4%)
Male	17 (77·3%)	12 (54·5%)
Age, years	28·0 (23·3, 32·8)	28·0 (24·0, 42·5)
	18·0 – 50·0	19·0 – 50·0
Height, cm	169·0 (160·8, 173·5)	170·0 (163·0, 179·0)
	157·0 – 189·0	159·0 – 192·0
Body weight, kg	65·5 (60·0, 75·0)	76·0 (59·0, ,80·0)
	42·0 – 115·0	43·0 – 100·0

*Table **S1***: **Demographic characteristics of subjects who participated in the phase 1 trial**
Data are presented as n (%), Median (IQR) and min-max range.

	Vaccine (n=22)	**Placebo (n=22)**
Any foreseen local and systemic AEs		
Any	7 (32%)*	0*
Mild (grade 1)	7 (32%)	0
Local reactions		
Pain	6 (27%)	0
Mild (grade 1)	6 (27%)	0
Systemic reactions		
Fever	1 (4·5%)	0
Mild (grade 1)	1 (4·5%)	0

*Table **S2***: **Safety of phase 1 clinical trial: local and systemic AEs observed within 7 days after the first immunisation**
Data are presented as n (%); subjects with more than one AE were only counted once. Adverse reactions were graded according to the Guidance for industry: Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (US Food and Drug Administration, 2007). *Attributable risk is 0.32 (95%CI 0·07-0·55), calculated by Newcombe/Wilson method with continuity correction

Assay	Placebo (n=22)				Vaccine (n=22)			
	Day 1	Day 21	Day 42	Day 180	Day 1	Day 21	Day 42	Day 180
MNA								
GMT (95% CI)	1·0 (1·, 1·0)	1·0 (1·0, 1·0)	1·0 (1·0, 1·0)	2 (1, 3)	1·0 (1·0, 1·0)	5·1 (3·5, 7·6)	100 (77, 129)	7 (5, 10)
Number with seroconversion	n/a	0/22	0/22	5/20†	n/a	13/22	22/22	22/22
% seroconversion (95% CI)	n/a	0 (0, 15)	0 (0, 15)	25 (11, 47)	n/a	59 (39, 77)	100 (85, 100)	100 (85, 100)
ELISA								
GMT (95% CI)	50 (50, 50)	52 (49, 55)	50 (50, 50)	230 (89, 593)	50 (50, 50)	1600 (1310, 1954)	30927 (19242, 49707)	454 (242, 852)
Number with seroconversion	n/a	0/22	0/22	8/20†	n/a	22/22	22/22	22/22
% seroconversion (95% CI)	n/a	0 (0, 15)	0 (0, 15)	40 (22, 61)	n/a	100 (85, 100)	100 (85, 100)	100 (85, 100)

Table S3: **Immunogenicity of phase 1 study: humoral immune response**
Data are GMTs and 95% CIs, % of subjects with antibody response is the percentage of subjects with ≥ fourfold antibody titre increase compared to Day 1, n/a – not applicable, † 2/22 subjects were lost to follow-up

Group	Day	Cytokine level				
		IFN-α	IFN-γ	IL-4	IL-6	TNF-α
Placebo	Day 1	11·4 (10·7, 13·1)	1·2 (1·1, 1·4)	1·0 (0·7, 1·1)	5·7 (4·9, 6·2)	0·6 (0·4, 0·7)
	Day 7	3·0 (1·9, 4·8)	0·2 (0·1, 0·3)	1·8 (1·3, 2·1)	1·2 (1·0, 1·9)	0·1 (0·1, 0·2)
	Day 21	16·5 (13·9, 19·6)	0·9 (0·6, 1·2)	1·9 (1·4, 2·6)	4·7 (3·5, 5·7)	0·4 (0·3, 0·7)
	Day 27	12·6 (10·3, 14·9)	0·5 (0·4, 0·6)	2·0 (1·4, 2·4)	4 ·0 (2·7, 4·2)	0·3 (0·2, 0·3)
	Day 42	14·8 (13·9, 18·1)	0·3 (0·3, 0·4)	1·7 (1·4, 2·0)	4·3 (3·7, 4·6)	0·2 (0·2, 0·2)
	Day 180	2·7 (1·5, 5·0)	0·5 (0·3, 3·2)	1·2 (1·0, 1·3)	2·5 (1·4, 12·5)	0·2 (0·1, 0·4)
Vaccine	Day 1	13·4 (11·4, 15·6)	1·3 (1·2, 1·4)	1 ·0 (0·8, 1·1)	5·2 (4·9, 5·5)	0·6 (0·5, 0·6)
	Day 7	50·7 (46·3, 53·7)	12·7 (9·4, 14·3)	2 (1·5, 2·5)	30·5 (27·4, 33·9)	3·9 (3·1, 4·3)
	Day 21	818·8 (597·2, 959·0)	25·2 (20·8, 34·3)	3·6 (2·9, 4·3)	141·6 (113·8, 173·6)	12·7 (8·5, 15·7)
	Day 27	4162·8 (3866·3, 4336·3)	37 ·0 (33, 42·5)	5·3 (4·7, 6·0)	1741·5 (1663·1, 2244·5)	30·4 (23·8, 34·5)
	Day 42	4791 ·0 (4487·6, 5020·2)	40·2 (36·2, 46·6)	5·8 (5·0, 7·6)	2772·1 (1650·2, 3500·8)	26·2 (22·8, 33·0)
	Day 180	5·8 **** (0·8, 9·4)	5·9 **** (4·0, 10·5)	1·4 **** (1·1, 1·8)	23·7 **** (18·5, 32·0)	4·8 **** (3·0, 12·2)

Table **S4: Cytokine response on different time points from the Phase 1 study**
Data are group Median (with IQR) cytokines levels measured in pg/ml. **** $p < 0{\cdot}0001$ indicates statistically significant decrease of cytokine levels in the vaccine group on Day 180 in comparison to Day 42, estimated in Bonferroni's multiple comparison test after two-way RM-ANOVA.

	18 – 49 years				**≥ 50 years**			
	Day 21	Day 27	Day 42	Day 180	Day 21	Day 27	Day 42	Day 180
Double vaccination								
Titre fold change (95% CI)	29 (16, 54)	79 (42, 147)	**205 (112, 374)**	37 (20, 68)	34 (20, 60)	100 (54, 184)	**205 (109, 385)**	56 (26, 121)
Single vaccination								
Titre fold change (95% CI)	16 (9, 29)	40 (21, 75)	70 (37, 130)	27 (11, 66)	21 (11, 38)	45 (24, 87)	67 (33, 133)	9 (4, 22)

Table S5: **Humoral immune response in phase 2 (ELISA results)**
Presented are geometric means of antibody titre fold change at indicated time points relative to Day 1 with 95% CIs calculated from assumed normal distribution of corresponding logarithmic values.