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Safety and immunogenicity of an inactivated virus particle vaccine for SARS-CoV-2, BIV1-CovIran: findings from double-blind, randomised, placebo-controlled, phase I and II clinical trials among healthy adults

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ABSTRACT

Objective Assessing safety and immunogenicity of an inactivated whole virus particle vaccine.

Design Single-centre, double-blind, randomised, placebo-controlled, phase I (stage I: 18–50, stage II: 51–75 years), phase II (18–75 years) clinical trials.

Setting 29 December 2020 to 22 April 2021.

Participants Stage I-phase I: 56 participants; stage II-phase I: 32; phase II: 280.

Intervention During stage I, participants randomly (3:3:1) received 3 µg, 5 µg vaccine or placebo in a 14-day interval. Participants in stage II received two shots of 5 µg vaccine or placebo (3:1). In phase II, participants received 5 µg vaccine or placebo (4:1) in a 28-day interval.

Primary and secondary outcome measures Safety assessment and immunogenicity assessment via antibody response and conventional virus neutralisation test (cVNT).

Results All adverse events (AEs) were mild or moderate and transient in both phase I and phase II, and no AEs of special interest were reported. The seroconversion-rate of neutralising, antireceptor binding-domain (RBD) and anti-spike-glycoprotein (anti-S) antibodies 14-days after second dose of 5 µg vaccine in stage I was 70.8% (95% CI 48.9% to 87.4%), 87.5% (95% CI 67.6% to 97.3%), 91.7% (95% CI 73.0% to 99.0%). The antibody titres increased more among 5 µg than 3 µg. The corresponding rates for 3 µg vaccine were 45.8% (95% CI 25.6% to 67.2%), 54.2% (95% CI 32.8% to 74.5%) and 70.8% (95% CI 48.9% to 87.4%), respectively. In stage II, 100% (95% CI 84.6% to 100%), 86.4% (95% CI 65.1% to 97.1%) and 86.4% (95% CI 65.1% to 97.1%) of participants seroconverted for neutralising, anti-RBD and anti-S antibodies. In phase II, the seroconversion rate of neutralising-antibody was 82.8% (95% CI 77.0% to 87.6%), anti-RBD 77.0% (95% CI 70.7% to 82.6%) and anti-S 79.9% (95% CI 73.8% to 85.1%) on day 42. In the cVNT, the sera at 1/64 times dilution would neutralise SARS-CoV-2 among 91.7%, 77.3% and 82.5% of vaccinated participants in phase I-stage I, phase I-stage II and phase II clinical trials, respectively.

Conclusions These results support further evaluation of this inactivated whole virus particle vaccine.

Trial registration numbers IRCT20201202049567N1 and IRCT20201202049567N2 for phase I and IRCT20201202049567N3 for phase II.

Strengths and limitations of this study

⇒ Antibody response was assessed via determining the geometric mean titres and the seroconversion rates of neutralising, antireceptor binding-domain and anti-spike-glycoprotein antibodies in both phases.
⇒ The conventional virus neutralisation test was performed to evaluate the levels of functional antibodies raised against SARS-CoV-2.
⇒ Cellular immunity induced by vaccination was not assessed in the study.

INTRODUCTION

A tremendous global effort has been made to rapidly produce vaccines against SARS-CoV-2 as a strategy to control the COVID-19 pandemic. Experts believe that safe and effective vaccines may be a potential pathway for controlling this ongoing crisis.[1 2] Remarkably, the time between identifying SARS-CoV-2 as an emerging pathogen and completing the first clinical trial for a vaccine was less than 9 months.[2 3]

As of 3 August 2021, 294 vaccines were being studied, among which 110 vaccines have been tested on humans in clinical trials.[4]

Fortunately, several COVID-19 vaccines showed promising results in phase 3 clinical trials, and vaccinations began in early 2021.[5 6] WHO has authorised emergency use for six vaccines and continues to evaluate additional proposals.[7] Nevertheless, since the introduction of vaccines against SARS-CoV-2 of various platforms worldwide, a growing body of literature has been focusing on vaccine safety,[8] efficacy[9] and their estimated effectiveness[10] against infection, symptomatic and severe disease caused by SARS-CoV-2 variants, and how the effectiveness wanes over time.[11]

Notwithstanding such impressive achievements, the production and distribution of billions of vaccine doses around the globe remain challenging. There are concerning inequities regarding timely access to safe COVID-19 vaccine, as only 1% of available vaccine doses worldwide have been administered in Africa. The COVID-19 Vaccines Global Access (COVAX) scheme has endeavoured to ensure fair access to vaccines, as no one is safe until everyone is safe. Nevertheless, COVAX has not progressed as expected due to the lack of support from wealthy nations and significant vaccine production challenges.[12]

COVID-19 has resulted in more than 4 million reported cases and 93 thousand confirmed deaths in Iran on 6 August 2021.[13] Since the beginning of the crisis, the Iranian healthcare system has faced limited access to life-saving medicines and equipment.[14] As of 6 August 2021, less than 3.5% of the Iranian population have been fully vaccinated for COVID-19.[13] Considering that some 60 million adults in Iran need vaccination,[15] the prompt administration of a safe domestic COVID-19 vaccine could be valuable in controlling the crisis and preventing the spread of new mutations of SARS-CoV-2.

Considering Iran's successful experiences in the mass-production of inactivated vaccines,[16] efforts to make domestic vaccines of this platform against SARS-CoV-2 seemed feasible. BIV1-CovIran is an inactivated whole virus particle vaccine that has demonstrated safety and immunogenicity in preclinical studies in mice, rabbits and non-human primates[17]; therefore, it was approved for progression to human studies. This study presents the results of phase I and II randomised placebo-controlled clinical trials of the BIV1-CovIran vaccine to assess its safety and immunogenicity.

METHODS

This study reports the findings of single-centre, double-blind, randomised, phase I and Phase II clinical trials of BIV1-CovIran vaccine among adults aged 18–75. Participants, outcome assessors, data managers, statisticians and other study-related personnel were masked to group allocations. Two intramuscular doses of the vaccine were administered on days 0 and 14 in phase I and days 0 and 28 in phase II. The primary outcomes included the safety assessment of the vaccine in phase I and the immunogenicity induced by the vaccine administration in phase II. The study protocol is presented in online supplemental appendix 1.

Study design

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The study protocol was fully explained to volunteers at screening, and all participants provided written informed consent before enrolment.[18–20] An independent data and safety monitoring board (DSMB) periodically evaluated the data and advised the outcome assessors about the clinical trials' continuation, suspension or early termination.

Phase I and II were conducted as single-centre, randomised, placebo-controlled, parallel-designed, double-blind clinical trials to evaluate the inactivated whole virus particle vaccine's safety, tolerability and immunogenicity BIV1-CovIran. Phase I was carried out in two stages: Stage I included individuals aged 18–50, and stage II included individuals aged 51–75 years.

Setting

The first vaccine/placebo injection of the first participant in stage I of phase I occurred on 29 December 2020, and the last dose was administered on 4 March 2021. The first vaccine/placebo injection of the first participant in stage II of phase I occurred on 15 March 2021, and the last dose was administered on 9 April 2021. The first vaccine/placebo injection of the first participant in phase II occurred on 15 March 2021, and the last dose was administered on 25 May 2021. Notably, the recruitment of participants aged 51–75 in phase II started on 22 April 2021, after initial safety analysis of the corresponding age group in phase I (figure 1). The study site, where enrolment, injections, participant monitoring and follow-up visits took place, was Eram Hotel, Tehran, Iran.

Patient and public involvement statement

The public was not involved in setting the research question, the outcome measures, the design or implementation of the study.

Participants

Invitations to participate were shared on mass media and social media platforms, and volunteers were contacted and then received detailed explanations about the clinical trial protocol. A pre-enrolment screening was conducted at the clinical trial site, including medical history, physical examination and laboratory tests. Participants aged 18–75 years who did not have a history of COVID-19, documented via medical history and negative serological screening, and were not infected with SARS-CoV-2 at the time of screening, documented via a negative real-time reverse transcription PCR (RT-PCR), the absence of suspicious symptoms, and no contact with a person with confirmed SARS-CoV-2 infection in the past 14 days, were included. The serological screening was performed using ELISA kits: PT-SARS-CoV-2.IgM-96 (the reported sensitivity and specificity: 79.4% and 97.30%, respectively)[21] and PT-SARS-CoV-2.IgG-96 (the reported sensitivity and

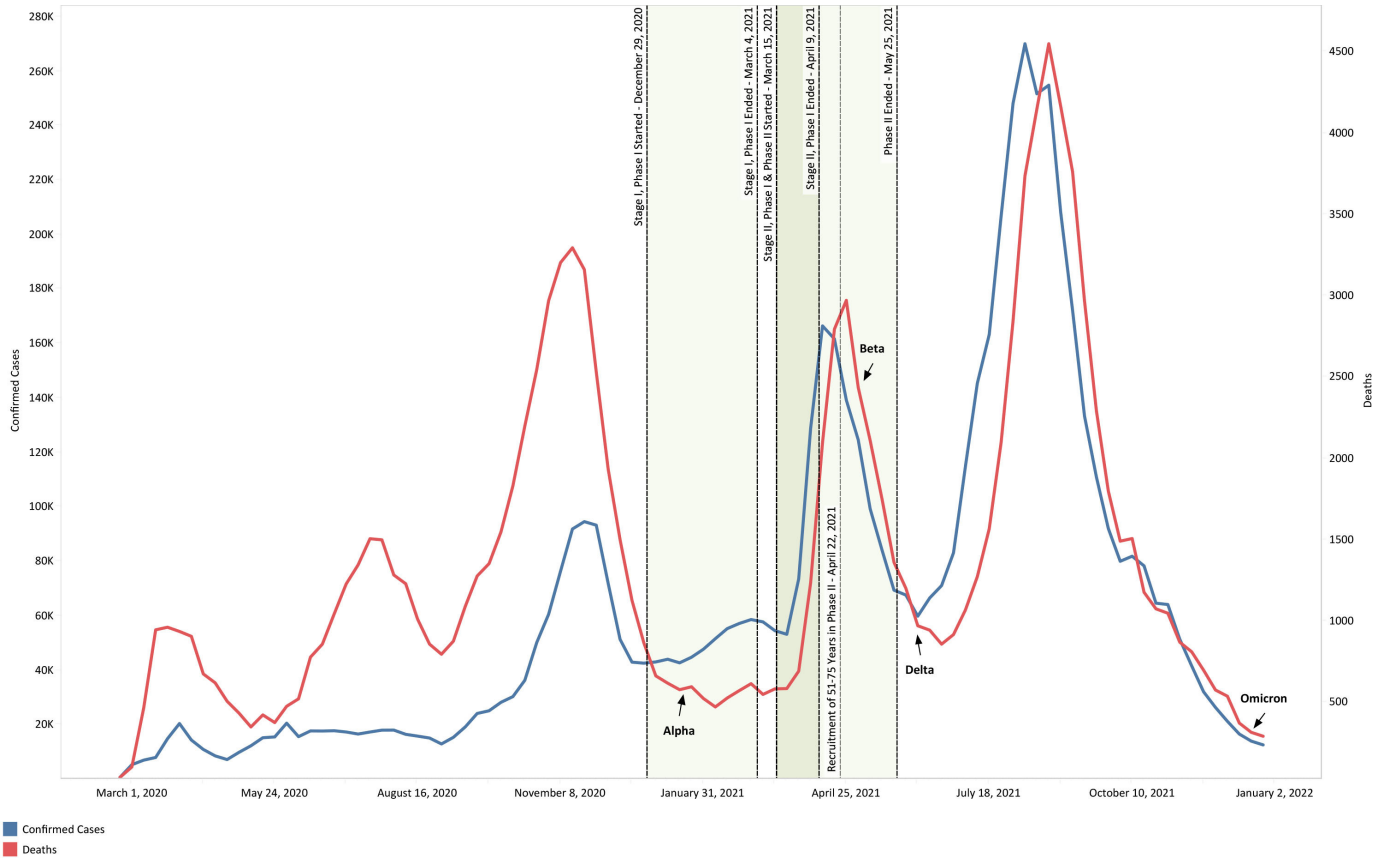

Figure 1 Mapping the timeline of phase I and phase II clinical trials with the time trend of COVID-19 weekly new cases (blue line) and mortality (red line) in Iran by the first week of January 2022.

specificity: 91.1% and 98.3%, respectively),[22] Pishtaz Teb,[23] Tehran, Iran.

In phase I, volunteers with increased risk for severe COVID-19 were excluded. During phase II, volunteers with any uncontrolled diseases like uncontrolled blood pressure (systolic and diastolic blood pressure above 140 and 90 mmHg, respectively), diabetes, chronic heart, kidney, liver, neurological or pulmonary diseases in medical examinations and according to the volunteer history (significant change in the course of treatment or hospitalisation due to exacerbation of the disease in the last 3 months) were excluded. However, like other healthy individuals, all patients with controlled, mild to moderate diseases could attend phase II of the study. Other key exclusion criteria included a self-reported history of severe allergic reactions, known allergy to vaccine ingredients, genetic, congenital or neurological disorders, chronic renal, hepatic or pulmonary diseases, malignancy, immunodeficiency, coagulation abnormalities, tuberculosis and hepatitis B or C. Pregnant or breastfeeding volunteers, women who had an intention to get pregnant in the following year, and those who did not plan to use contraception during the study period were also excluded. Receiving a live attenuated vaccine in the prior month, or any vaccines in the past 14 days, as well as receiving immunosuppressive medication, immunoglobulin or blood products during the past 3 months, led to exclusion from the clinical trial. Notably, participants were advised to delay other live or attenuated vaccine injections up to at least 1 month after receiving the last dosage of the vaccine; however, exceptions were considered in case of an urgent indication for vaccination, such as for rabies postexposure prophylaxis. Individuals with occupations that were deemed high risk for SARS-CoV-2 exposure (eg, healthcare professionals) did not enter the study. Further details about screening and eligibility criteria are available in the summary of study protocols.[18–20]

Enrolment, randomisation and interventions

Phase I

In stage I, a total of 56 volunteers aged 18–50 years were randomised with an allocation ratio of 3:3:1 into three arms to receive 3 µg of the vaccine (24 participants), 5 µg of the vaccine (24 participants) or placebo (8 participants) on days 0 and 14. Randomisation was conducted in two stages. Initially, 14 participants were randomised to receive the 3 µg dosage of the vaccine or placebo (12 vs 2). Participants were monitored for 7 days after injection, followed by a DSMB meeting that approved the vaccine safety and authorised further proceeding. The remaining 42 individuals were randomised to the 3 µg, 5 µg, and placebo arms. The randomisation sequence was generated by a computer in a block size of 7. Two types of randomisation blocks were used, corresponding to the two randomisation steps. The first two blocks allocated six participants

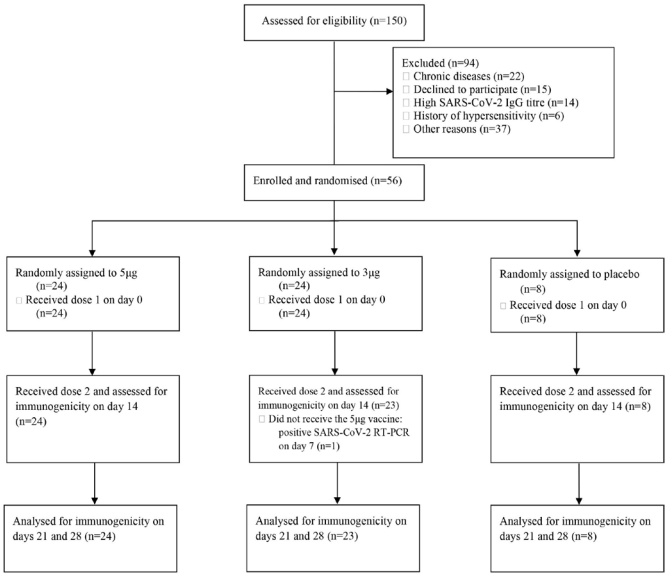

Figure 2 Diagram of screening, enrolment, randomisation and follow-up in stage I of phase I. RT-PCR, reverse transcription PCR.

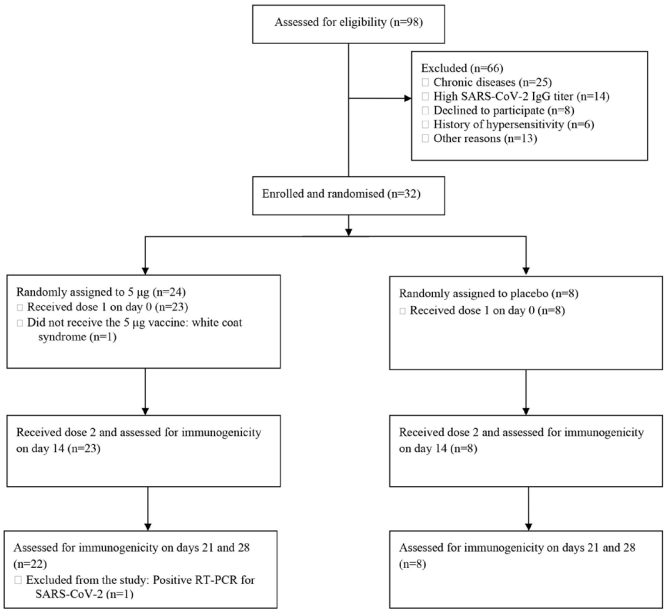

Figure 3 Diagram of screening, enrolment, randomisation and follow-up in stage II of phase I. RT-PCR, reverse transcription PCR.

to the 3 µg vaccine group and one to the placebo group. The remaining six blocks were randomised with an allocation ratio of 2:4:1, in which participants were assigned to three study groups: 3 µg of vaccine, 5 µg of vaccine or placebo, respectively (figure 2).

In stage II of phase I, after a review of the safety and immunogenicity data of this age group by the DSMB, 32 volunteers aged 51–75 years were enrolled to randomly receive 5 µg of the vaccine (24 participants) or placebo (eight participants) on days 0 and 14. The 5 µg dose was favoured over 3 µg due to better immunogenicity based on the interim analysis of stage I. The randomisation sequence was computer generated in permuted blocks of size 4 with an allocation ratio of 3:1. All random allocation processes were performed by an interactive web response system (figure 3).

Phase II

In phase II, the vaccine schedule was modified to enhance efficacy, based on the experts' opinion after early results of phase I, as well as the emerging evidence from other studies.[24–26] Thus, the intervention arm received 5 µg of the vaccine on days 0 and 28; volunteers in phase II were stratified based on their age group—age 18–50 and 51–75 years. Participants aged 51–75 years were not recruited in phase II, until safety results from that age group in phase I were available. Overall, 280 participants (200 aged 18–50 years and 80 aged 51–75 years) were randomised with a 4:1 ratio to receive 5 µg vaccine shots (224 participants) or a placebo (56 participants), as presented in figure 4.

In both phases of the study, a 0.5 mL dose of vaccine/placebo was administered intramuscularly into the deltoid muscle of the non-dominant side. After receiving the first dosage, individuals who experienced a severe allergic reaction, severe fever (axillary temperature ≥39°C) for 3 days, or other vaccine-related serious adverse events (SAEs), and participants with positive RT-PCR after the first dose would not proceed to receive the second dose. All vaccine and placebo vials containing one dose were identical in appearance and were labelled with a randomisation code by a contract research organisation (CRO). Access to each vial was authorised after finalising the enrolment of each eligible volunteer. Participants, outcome assessors, data managers, statisticians and other study-related personnel were blinded in allocation stages, vaccine injection and outcome assessment. Only the CRO was unblinded at the study site.

Procedures

BIV1-CovIran is an inactivated whole virus particle vaccine manufactured by Shifa Pharmed Industrial Group. The SARS-CoV-2 virus was isolated from the nasopharyngeal specimen of an Iranian patient with COVID-19. The virus was sequenced and cultured using a Vero cell manufacturing platform in a biosafety level 3 facility.[27] Viral particles were inactivated with β-propiolactone. After purification, the inactivated virus particles were sterilised with filtration and formulated with Alhydrogel as adjuvant (Croda International[28]). Each dose of vaccine included a maximum of 500 µg of alhydrogel.

Further details about vaccine production are presented elsewhere.[17] The placebo solution contained the same amount of Alhydrogel, diluted by phosphate-buffered saline. Vaccine and placebo vials were stored at 2°C–8°C.

Follow-up

Phase I

In phase I, participants resided in the clinical trial site (Eram Hotel) for up to 7 days after each injection for close observation. In this period, twice daily clinical visits by physicians and constant monitoring by study nurses were

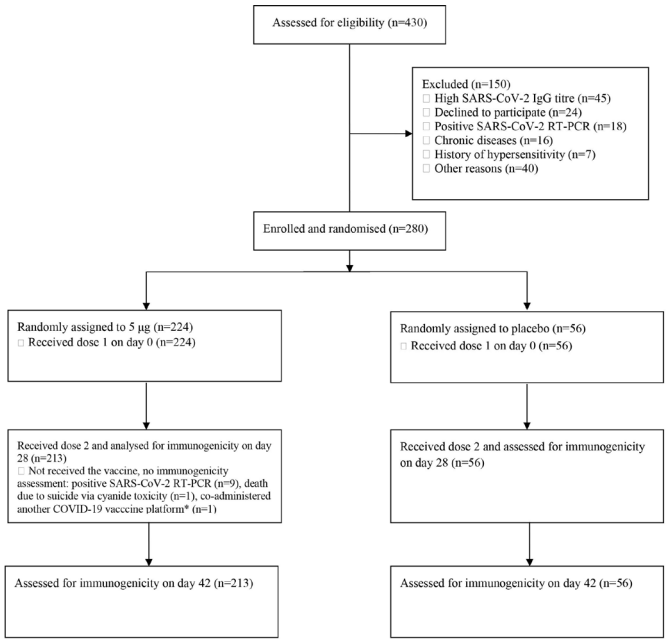

Figure 4 Diagram of screening, enrolment, randomisation and follow-up in phase II. *The latter participant reported administering a dose of another vaccine after the first injection of BIV1-CovIran, and was thus excluded from the study. RT-PCR, reverse transcription PCR.

provided to assess any AEs. On home discharge, participants were instructed to record their symptoms at home and fill out diary cards designed for this purpose. Moreover, follow-up phone calls by study nurses were made daily. On day 14, the second vaccine dose was administered at the clinical trial site, and participants were monitored for another 7 days in the hotel. On day 21, the physician visited participants and then would leave the trial site. Another follow-up visit occurred on day 28. In the meantime, participants were instructed to contact the 24/7 study call centre should they have any concerns or need medical attention. In case of suspicion for COVID-19, a nasopharyngeal specimen would be obtained at the clinical trial site, and RT-PCR would be performed at a central laboratory. Suspected COVID-19 cases were defined as presenting at least two of the following symptoms: fever (axillary temperature ≥37.5°C), chills, sore throat, stuffy nose, myalgia, fatigue, headache, nausea or vomiting, or diarrhoea; OR at least one respiratory sign or symptom (including cough, shortness of breath), new olfactory or taste disorder, radiographic evidence of COVID-19 like pneumonia. Blood samples were collected on days 7, 14, 21 and 28 after the first injection.

Phase II

In phase II, participants were monitored at the clinical trial site for at least an hour after injection. Visits were performed on day 28 (injection of the second dose) and day 42. Follow-up phone visits by study nurses were conducted at 14-day intervals. Participants were provided with diaries and instructed to record AEs or prespecified symptoms associated with COVID-19 infection. Moreover, participants would contact the 24/7 study call centre should they have any concerns or need medical attention. In case of suspicion for COVID-19, a nasopharyngeal specimen would be obtained at the clinical trial site, and RT-PCR would be performed at a central laboratory. Blood samples were collected on days 28 and 42 after the first injection.

Outcomes

Safety

The safety outcome was the incidence of any AEs after injections. The adverse events of special interest (AESI) defined for COVID-19 vaccines were investigated in the study.[29] The Food and Drug Administration Guidance for Industry and Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials[30] were used for AEs categorisation. Any other AEs not mentioned in the guidance were classified based on the Common Terminology Criteria for Adverse Events V.5.0.[31] Solicited AEs were defined as any events which occurred from day zero to day seven after each injection. Unsolicited AEs were defined as any AEs which occurred from day 8 to day 28 after each injection. All events were classified based on the Medical Dictionary for Regulatory Activities, V.23.1, and are reported irrespective of the causality.[32]

Immunogenicity

Immunogenicity outcomes were categorised based on humoral responses to the vaccine. The humoral response was assessed through geometric mean titres (GMT), geometric mean ratios (GMR) of antibodies against SARS-CoV-2, and seroconversion rate. GMR was defined as the ratio of GMTs in the vaccine group to the corresponding titres in the placebo group at the same time point. Seroconversion was defined as an increase in antibodies ≥4 times their baseline level. Neutralising, antireceptor binding domain (RBD) and anti-spike glycoprotein antibodies were measured using ELISA kits: SARS-CoV-2 Neutralising Ab IgG-96,[33] SARS-CoV-2 RBD IgG-96[34] and SARS-CoV-2 spike IgG-96,[35] Pishtaz Teb, Tehran, Iran. Moreover, antibodies against S1 domain of the spike glycoprotein of SARS-CoV-2 were assessed via EI 2606-9601 G kit, Euroimmun.[36]

Conventional virus neutralisation test assay

Conventional virus neutralisation test (cVNT) was employed to assess the vaccine effectiveness in inducing functional antibodies against SARS-CoV-2. To inactivate the complement, plasma samples were heated at 56°C for 30 min. Afterwards, plasma samples were serially diluted in two-fold dilutions. SARS-CoV-2 suspensions at 100 Tissue Culture Infectious Dose 50 assay (TCID50) were incubated with diluted plasma at 37°C and 5% CO2 for an hour. Monolayer Vero E6 cells with 80% confluency were overlaid with plasma-virus suspensions. Each neutralisation test was performed in triplicates. Then, virus-specific cytopathic effects (CPE) were visualised

72 hours later and were observed via light microscopy. The Reed-Muench method was applied to calculate the neutralising antibody titre that reduced the number of infected wells by 90%.[37 38] Neutralising antibody titres are presented as values of the highest dilution inhibiting CPE formation.[39 40]

Statistical analysis

The sample size was not determined based on the statistical power calculation. The ratio of vaccination to placebo was 3:3:1, containing 3 µg or 5 µg whole virus particle or placebo, in stage I-phase I; 4:1, containing 5 µg whole virus particle or placebo, in stage II-phase I; and 3:1, containing 5 µg whole virus particle or placebo, in phase II. The safety analysis was conducted for all participants who received at least one dose of the vaccine/placebo after randomisation and had any safety evaluation data. The incidence of AEs in each subgroup was defined as the number of participants with AEs divided by the number of participants in the corresponding intervention/placebo subgroup. The analysis of humoral immunogenicity was conducted for all enrolled participants who had randomly received the vaccine/placebo with blood collection before and after each injection.

Frequency, mean and SD were used to describe the data. We used the χ^2 test and Fisher's exact test for categorised variables. D'Agostino's K-squared test was employed to check the normality of the distribution.[41] F-test of equality of variances was used to verify the equality of variances for the two-sample t-test.[42] If the normality assumption was not satisfied, the means were compared using the Mann-Whitney test. In cases of normal distribution, if the variances were equal, the mean titres among groups were compared with a two-sample t-test at a two-sided 5% significance level. Otherwise, the Welch correction (Welch's t-test) was used while using the two-sample t-test. Cramér's V was used to investigate the effect size for the safety analysis.[43]

The statistical analyses were carried out using R statistical packages V.3.4.3 (http://www.r-project.org, RRID: SCR_001905). Data visualisations were performed using Tableau Desktop, V.2020.1, an interactive data visualisation software. Data for visualisation of weekly COVID-19 new cases and mortality in figure 1 were derived from an interactive web-based dashboard to track COVID-19 in real time.[44]

Role of the funding source

The study's sponsor was not involved in study design and had no role in data collection, analysis, interpretation, manuscript drafting or submission. Clinical Trial Center (CTC), an academic CRO affiliated with Tehran University of Medical Sciences, Tehran, Iran, performed clinical trial management and monitoring. The unmasked randomisation list was not shared with the study sponsor. Data cleaning, analysis, and drafting the manuscript were done by a third-party research centre (Non-Communicable Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran).

RESULTS

Phase I

As many as 56 participants were enrolled in stage I and 32 in stage II of phase I (table 1). None of the participants in phase I had any underlying conditions. Figures 2–4 demonstrate diagrams of screening, enrolment, randomisation and follow-up at phase I.

Safety

Among participants aged 18–50 years, the overall incidence of solicited AEs after the first injection was 14/24 (58.3%) in the 3 µg group, 16/24 (66.7%) in the 5 µg group and 6/8 (75.0%) in the placebo group. The overall incidence of local solicited AEs after the first injection in stage I was 16/56 (28.6%), including 6/24 (25.0%) in the

Table 1 Baseline characteristics of participants in phase I clinical trial

Characteristics	Stage I (18–50 years)			Stage II (51–75 years)	
	Placebo (N=8)	3 µg (N=24)	5 µg (N=24)	Placebo (N=8)	5 µg (N=24)
Sex (N, %)					
Female	4 (50.0)	10 (41.7)	6 (25.0)	4 (50.0)	11 (45.8)
Male	4 (50.0)	14 (58.3)	18 (75.0)	4 (50.0)	13 (54.2)
Age (mean-SD)	34.4 (7.8)	34.0 (8.6)	35.0 (6.8)	55.5 (3.5)	58.5 (6.9)
Baseline vital signs (mean-SD)					
Body temperature (°C)	36.8 (0.4)	36.6 (0.2)	36.5 (0.2)	36.7 (0.2)	35.2 (7.5)
Respiratory rate (per minute)	16.1 (1.3)	15.9 (1.0)	15.6 (0.5)	15.3 (0.9)	14.5 (3.2)
Heart rate (beats per minute)	89.9 (3.1)	82.9 (9.3)	86.7 (6.8)	81.0 (12.9)	79.0 (18.9)
Systolic blood pressure (mm Hg)	120.6 (11.6)	119.7 (11.7)	118.6 (10.6)	124.9 (8.5)	120.0 (26.3)
Diastolic blood pressure (mm Hg)	79.6 (10.4)	77.2 (9.6)	77.5 (6.5)	81.5 (4.0)	77.8 (17.0)

3 µg, 8/24 (33.3%) in the 5 µg and 2/8 (25.0%) in the placebo group. In addition, 31/56 (55.4%) participants showed systemic solicited AEs: 13/24 (54.2%) participants in the 3 µg, 13/24 (54.2%) in the 5 µg and 5/8 (62.5%) in the placebo group. Of 12/56 (21.4%) participants who had unsolicited AEs after the first injection, 6 were in 3 µg group, 3 were in 5 µg group and 3 in the placebo group. In stage I, there were low significant differences in the incidence ratio of solicited (Cramér's V=0.46) and unsolicited (Cramér's V=0.36) AEs between the intervention and placebo groups.

Considering the exclusion of one participant in the 3 µg group due to a positive RT-PCR test, of all 55 participants who received the second injection, 38/55 (69.1%) and 9/55 (16.4%) showed solicited and unsolicited AEs, respectively. Among participants with solicited AEs after the second injection, 14/23 (60.9%) were among the 3 µg group, 18/24 (75.0%) among the 5 µg group and 6/8 (75.0%) among the placebo group. The incidence of unsolicited AEs after the second injection was 4/23 (17.4%), 3/24 (12.5%), and 2/8 (25.0%) among 3 µg, 5 µg and placebo groups, respectively (online supplemental appendix 2, table S1).

A total number of 63 AEs occurred among 24/31 (77.4%) participants in stage II. Of 31 participants, 15 (48.4%) had at least one AE after the first injection and 19/31 (61.3%) after the second injection. As many as 15/31 (48.4%) participants in stage II had solicited AEs after the first injection and 18/31 (58.1%) after the second injection. The incidence of solicited AEs in the 5 µg group was 13/23 (56.5%) after the first injection and 14/23 (60.9%) after the second injection. After both injections, only 3/31 (9.7%) participants reported unsolicited AEs, all of them were in the intervention group (online supplemental appendix 2, table S2). Similar to stage I, there were low significant differences in the incidence ratio of solicited (Cramér's V=0.04) and unsolicited (Cramér's V=0.18) AEs between intervention and placebo groups.

Among participants of both stages, the most prevalent AE was pain at the injection site, followed by weakness and headache (online supplemental appendix 2, tables S1 and S2). All AEs among the vaccinated participants in phase I were mild or moderate, and no AESI was witnessed. There were low significant abnormalities in the laboratory assessment of participants during phase I (online supplemental appendix 2, tables S3 and S4).

In phase I, there were two SAEs, which occurred among men aged 44 and 63 years. The 44-year-old man had received the 3 µg vaccine, and his chief complaint was moderate chest discomfort 2 days after his first injection, with a normal ECG, creatine phosphokinase, high-resolution CT scan and negative COVID-19 RT-PCR. He was hospitalised in a general ward for one night, not needing significant medical interventions. He recovered and was discharged from the hospital symptom-free 1 day later. The investigator considered the event unrelated to the intervention, and the participant proceeded to the second injection without any problems.

The 63-year-old man had coughing, myalgia and mild headache before the second injection of the 5 µg vaccine. The evaluation of vital signs was normal, the COVID-19 RT-PCR test result was negative on symptom onset, and there were no signs of lung involvement in the physical examination. Thus, he received the second dose of the vaccine. Following close observation and during the daily physical examinations, the symptoms exacerbated and the outcome assessors decided to repeat the COVID-19 RT-PCR test, which turned out to be positive. The essential diagnostic and therapeutic measures were instantly taken, and he was admitted to the hospital due to moderate bradycardia. Ten days later, he recovered and was discharged from the hospital. Similar to the first event, the investigator defined the causality assessment of the event as unrelated to the vaccine.

Immunogenicity

At the baseline, none of the participants was positive for SARS-CoV-2 RT-PCR, nor did they have any detectable IgM or IgG antibodies against SARS-CoV-2. All anti-SARS-CoV-2 antibodies increased over time after the second injection of the vaccine. Neutralising antibody increased on day 21 in all vaccine groups; however, the antibody level continued the sharp increase on day 28 in the 5 µg group, while it plateaued in the 3 µg group. Similarly, anti-spike glycoprotein antibody rose sharply by day 21 in both 3 µg and 5 µg groups. Nevertheless, anti-RBD antibody continued to increase until day 28 with the 5 µg vaccine, while it plateaued in the 3 µg group after day 21 (table 2). GMR of neutralising, anti-RBD, and anti-spike glycoprotein antibodies at different time points in phase I is presented in table 3.

Among participants aged 18–50 years, the seroconversion rate (95% CIs) of neutralising antibodies 14 days after the second dose of vaccine injection was 45.8% (25.6% to 67.2%) in the 3 µg group, 70.8% (48.9% to 87.4%) in the 5 µg group and 37.5% (8.5% to 75.5%) in the placebo group. Simultaneously, the seroconversion rate of anti-RBD antibodies (95% CI) was 54.2% (32.8% to 74.5%) in the 3 µg group, 87.5% (67.6% to 97.3%) in the 5 µg group, and 0.0 (0.0 to 0.0) in the placebo group. The seroconversion rate of anti-spike antibodies (95% CI) was 70.8% (48.9% to 87.4%) in the 3 µg group, 91.7% (73.0% to 99.0%) in the 5 µg group, and 50.0% (15.7% to 84.3%) in the placebo group (table 4, figure 5A–C). Anti-spike glycoprotein antibody was also assessed via Euroimmun kit, which showed a 91.7% (73.0% to 99.0%) seroconversion rate on day 28 in the 5 µg group (online supplemental appendix 2, table S5). In cVNT, the sera at 1/64 times dilution of 91.7% of vaccinated participants with 5 µg BIV1-CovIran neutralised SARS-CoV-2. In contrast, zero per cent of the participants' sera at the same dilution neutralised the virus in the placebo group (figure 6).

Table 2 Geometric mean titres of neutralising, anti-receptor-binding domain, and anti-spike glycoprotein antibodies at different time points in phase I

	Stage I (18–50 years)			Stage II (51–75 years)	
	Geometric mean titre* (95% CI)			Geometric mean titre (95% CI)	
Antibody	3 µg	5 µg	Placebo	5 µg	Placebo
Neutralising antibody					
Day 0	1.76 (1.37 to 2.26)	1.36 (0.87 to 2.11)	1.55 (1.20 to 2.01)	0.37 (0.28 to 0.48)	0.56 (0.41 to 0.76)
Day 14	2.46 (1.26 to 4.79)	2.76 (1.58 to 4.81)	2.36 (0.70 to 8.00)	0.92 (0.42 to 2.02)	0.31 (0.20 to 0.49)
Day 21	6.26 (3.08 to 12.71)	7.79 (3.61 to 16.80)	1.31 (1.08 to 1.60)	5.39 (2.69 to 10.83)	0.80 (0.46 to 1.40)
Day 28	7.89 (3.60 to 17.28)	15.38 (8.02 to 29.48)	2.76 (0.63 to 12.11)	12.52 (7.29 to 21.51)	0.85 (0.27 to 2.66)
Anti-receptor binding domain IgG					
Day 0	0.19 (0.10 to 0.37)	0.17 (0.10 to 0.29)	0.1 (0.1 to 0.1)	0.14 (0.10 to 0.21)	0.1 (0.1 to 0.1)
Day 14	0.37 (0.16 to 0.84)	2.24 (1.27 to 3.95)	0.1 (0.1 to 0.1)	0.30 (0.14 to 0.66)	0.1 (0.1 to 0.1)
Day 21	0.86 (0.38 to 1.95)	7.63 (5.18 to 11.22)	0.14 (0.08 to 0.28)	4.00 (1.84 to 8.71)	0.1 (0.1 to 0.1)
Day 28	1.23 (0.56 to 2.69)	7.58 (5.66 to 10.14)	0.12 (0.09 to 0.16)	6.02 (3.26 to 11.13)	0.1 (0.1 to 0.1)
Anti-spike glycoprotein IgG					
Day 0	0.85 (0.30 to 2.40)	0.26 (0.12 to 0.56)	0.11 (0.09 to 0.13)	0.33 (0.14 to 0.75)	0.27 (0.11 to 0.67)
Day 14	2.26 (0.67 to 7.58)	2.28 (0.89 to 5.84)	0.19 (0.08 to 0.46)	0.69 (0.24 to 1.98)	0.19 (0.10 to 0.34)
Day 21	10.19 (4.45 to 23.34)	54.84 (36.32 to 82.82)	0.68 (0.30 to 1.54)	19.56 (7.64 to 50.09)	0.17 (0.09 to 0.31)
Day 28	10.39 (4.17 to 25.88)	70.41 (55.01 to 90.13)	0.30 (0.13 to 0.73)	53.69 (29.09 to 99.10)	0.18 (0.09 to 0.40)

Results reported at baseline (day 0), 2 weeks after the first vaccination (day 14), and 2 weeks after the second vaccination (day 28) for 3 µg, 5µg, and placebo groups. In stage I, one participant in the 3 µg group became RT-PCR positive for COVID-19 on day seventh after the first dose and was thus excluded from the study. In stage II, one participant in the 5 µg group was excluded from the study and did not receive any doses due to white coat syndrome. Another participant in the 5 µg group of stage II became RT-PCR positive for COVID-19 within a day after the second injection and thus was excluded from data analysis.
*Neutralising antibody is reported in µg/mL—anti-receptor binding domain IgG in RU/mL—and anti-spike glycoprotein IgG in RU/mL.
RT-PCR, reverse transcription PCR.

Table 3 Geometric mean ratios of neutralising, anti-receptor-binding domain, and anti-spike glycoprotein antibodies at different time points in phase I

	Stage I (18–50 years)		Stage II (51–75 years)
	Geometric mean ratio* (95% CI)		Geometric mean ratio (95% CI)
Antibody	3 µg	5 µg	5 µg
Neutralising antibody			
Day 0	1.13 (0.72 to 1.77)	0.87 (0.4 to 1.89)	0.65 (0.41 to 1.04)
Day 14	1.04 (0.28 to 3.8)	1.17 (0.38 to 3.61)	2.93 (0.78 to 10.97)
Day 21	4.77 (1.4 to 16.33)	5.94 (1.57 to 22.51)	6.70 (2.05 to 21.94)
Day 28	2.86 (0.62 to 13.24)	5.58 (1.47 to 21.14)	14.81 (5.11 to 42.93)
Anti-receptor binding domain IgG			
Day 0	1.93 (0.63 to 5.93)	1.69 (0.66 to 4.35)	1.40 (0.74 to 2.65)
Day 14	3.71 (0.89 to 15.37)	22.39 (8.4 to 59.69)	3.03 (0.84 to 10.86)
Day 21	5.99 (1.4 to 25.62)	53.14 (25.4 to 111.19)	39.98 (11.05 to 144.58)
Day 28	10.34 (2.65 to 40.27)	63.7 (37.86 to 107.19)	60.23 (21.83 to 166.16)
Anti-spike glycoprotein IgG			
Day 0	7.91 (1.31 to 47.66)	2.37 (0.61 to 9.18)	1.23 (0.29 to 5.18)
Day 14	11.71 (1.38 to 99.25)	11.83 (2.21 to 63.39)	3.69 (0.63 to 21.75)
Day 21	14.92 (3.37 to 65.95)	80.32 (35.59 to 181.29)	117.17 (24.04 to 571.11)
Day 28	34.28 (6.69 to 175.63)	232.26 (127.12 to 424.36)	292.79 (98.91 to 866.70)

Results reported at baseline (day 0), 2 weeks after the first vaccination (day 14), and 2 weeks after the second vaccination (day 28) for 3 µg, 5 µg, and placebo groups. In stage I, one participant in the 3 µg group became RT-PCR positive for COVID-19 on day seventh after the first dose and was thus excluded from the study. In stage II, one participant in the 5 µg group was excluded from the study and did not receive any doses due to white coat syndrome. Another participant in the 5 µg group of stage II became RT-PCR positive for COVID-19 within a day after the second injection and thus was excluded from data analysis.
*Neutralising antibody is reported in µg/mL—anti-receptor binding domain IgG in RU/mL—and anti-spike glycoprotein IgG in RU/mL.
RT-PCR, reverse transcription PCR.

Among participants aged 51–75, the seroconversion rates of neutralising, anti-RBD and anti-spike glycoprotein antibodies at day 28 from the first injection in the 5 µg group were 100.0% (95% CI 84.6% to 100.0%), 86.4% (95% CI 65.1% to 97.1%) and 86.4% (95% CI 65.1% to 97.1%), respectively (table 4, figure 5D–F). Euroimmun anti-spike glycoprotein showed 77.3% (95% CI 54.6% to 92.2%) seroconversion rate on day 28 in the 5 µg group (online supplemental appendix 2, table S5). In cVNT, the sera at 1/64 times dilution of some 77.0% of vaccinated participants with 5 µg BIV1-CovIran neutralised SARS-CoV-2. In contrast, one-fourth of the participants' sera at the same dilution neutralised the virus in the placebo group (figure 6).

Phase II

Phase II clinical trial was conducted with the participation of 280 individuals: 224 in the 5 µg group and 56 in the placebo group. The mean (SD) age of participants was 42.2 (12.8) in the 5 µg group and 40.4 (12.4) in the placebo group (table 5). Figure 4 demonstrates diagrams of screening, enrolment, randomisation and follow-up in phase II.

Safety

A total number of 317 AEs occurred in 152/280 (54.0%) participants during phase II: 125/224 (56.3%) among the 5 µg group compared with 27/56 (46.4%) among the placebo group (p=0.23, Cramér's V=0.07). Almost all solicited and unsolicited AEs were mild in both 5 µg and placebo groups. In the 5 µg group, the overall incidence rate of solicited AEs was 68/224 (30.4%) participants after the first injection. After the first injection, eleven participants were excluded (figure 4); thus, the incidence rate of solicited AEs after the second injection was 54/213 (25.3%). Among 56 participants in the placebo group, the overall incidence rate of solicited AEs was 12/56 (21.4%) after the first injection and 18/56 (32.1%) after the second injection.

As many as 10/280 (3.6%) participants showed unsolicited AEs after the first injection: 9/224 (4.0%) in the 5 µg group and 1/56 (1.8%) in the placebo group. After the second injection, 37/269 (13.8%) participants had unsolicited AEs: 29/213 (13.6%) in the 5 µg group and 8/56 (14.3%) in the placebo group. There was no difference between the incidence rates of AEs among the intervention and the placebo groups for solicited (p=0.23, Cramér's V=0.07) and unsolicited (p=0.70, Cramér's V=0.03) AEs.

The most common AE among phase II participants was a pain in the injection site, which was reported in 45/224 (20.1%) participants after the first injection of the vaccine and 40/213 (18.8%) after the second injection of vaccine vs

Table 4 The proportion of patients with seroconversion for neutralising, anti-receptor-binding domain, and anti-spike glycoprotein antibodies in phase I

	Stage I (18–50 years)			Stage II (51–75 years)	
	Seroconversion rate* (95% CI)			Seroconversion rate (95% CI)	
Antibody	**3 µg**	**5 µg**	**Placebo**	**5 µg**	**Placebo**
Neutralising antibody					
Day 14	12.50 (2.66 to 32.36)	25.00 (9.77 to 46.71)	12.50 (0.32 to 52.65)	22.73 (7.82 to 45.37)	0 (0 to 0)
Day 21	33.33 (15.63 to 55.32)	58.33 (36.64 to 77.89)	0 (0 to 0)	77.27 (54.63 to 92.18)	12.50 (0.32 to 52.65)
Day 28	45.83 (25.55 to 67.18)	70.83 (48.91 to 87.38)	37.5 (8.52 to 75.51)	100 (84.56 to 100)	12.50 (0.32 to 52.65)
Anti-receptor binding domain IgG					
Day 14	16.67 (4.74 to 37.38)	75.00 (53.29 to 90.23)	0 (0 to 0)	22.73 (7.82 to 45.37)	0 (0 to 0)
Day 21	16.67 (4.74 to 37.38)	87.50 (67.64 to 97.34)	12.50 (0.32 to 52.65)	77.27 (54.63 to 92.18)	0 (0 to 0)
Day 28	54.17 (32.82 to 74.45)	87.50 (67.64 to 97.34)	0 (0 to 0)	86.36 (65.09 to 97.09)	0 (0 to 0)
Anti-spike glycoprotein IgG					
Day 14	25.00 (9.77 to 46.71)	66.67 (44.68 to 84.37)	12.50 (0.32 to 52.65)	18.18 (5.19 to 40.28)	0 (0 to 0)
Day 21	70.83 (48.91 to 87.38)	91.67 (73.00 to 98.97)	75.00 (34.91 to 96.81)	72.73 (49.78 to 89.27)	0 (0 to 0)
Day 28	70.83 (48.91 to 87.38)	91.67 (73.00 to 98.97)	50.00 (15.7 to 84.3)	86.36 (65.09 to 97.09)	12.50 (0.32 to 52.65)

Results reported as the proportion of participants with at least fourfold higher seroconversion than the baseline titre at 2 weeks after the first vaccination (day 14), 3 weeks after the first vaccination (day 21), and 2 weeks after the second vaccination (day 28) for 3 µg, 5 µg, and placebo groups. In stage I, one participant in the 3 µg group became RT-PCR positive for COVID-19 on day seventh after the first dose and was thus excluded from the study. In stage II, one participant in the 5 µg group was excluded from the study and did not receive any doses due to white coat syndrome. Another participant in the 5 µg group of stage II became RT-PCR positive for COVID-19 within a day after second injection and thus was excluded from data analysis.
**Defined as a postvaccination titre that was at least four-fold higher than the baseline titre.
RT-PCR, reverse transcription PCR.

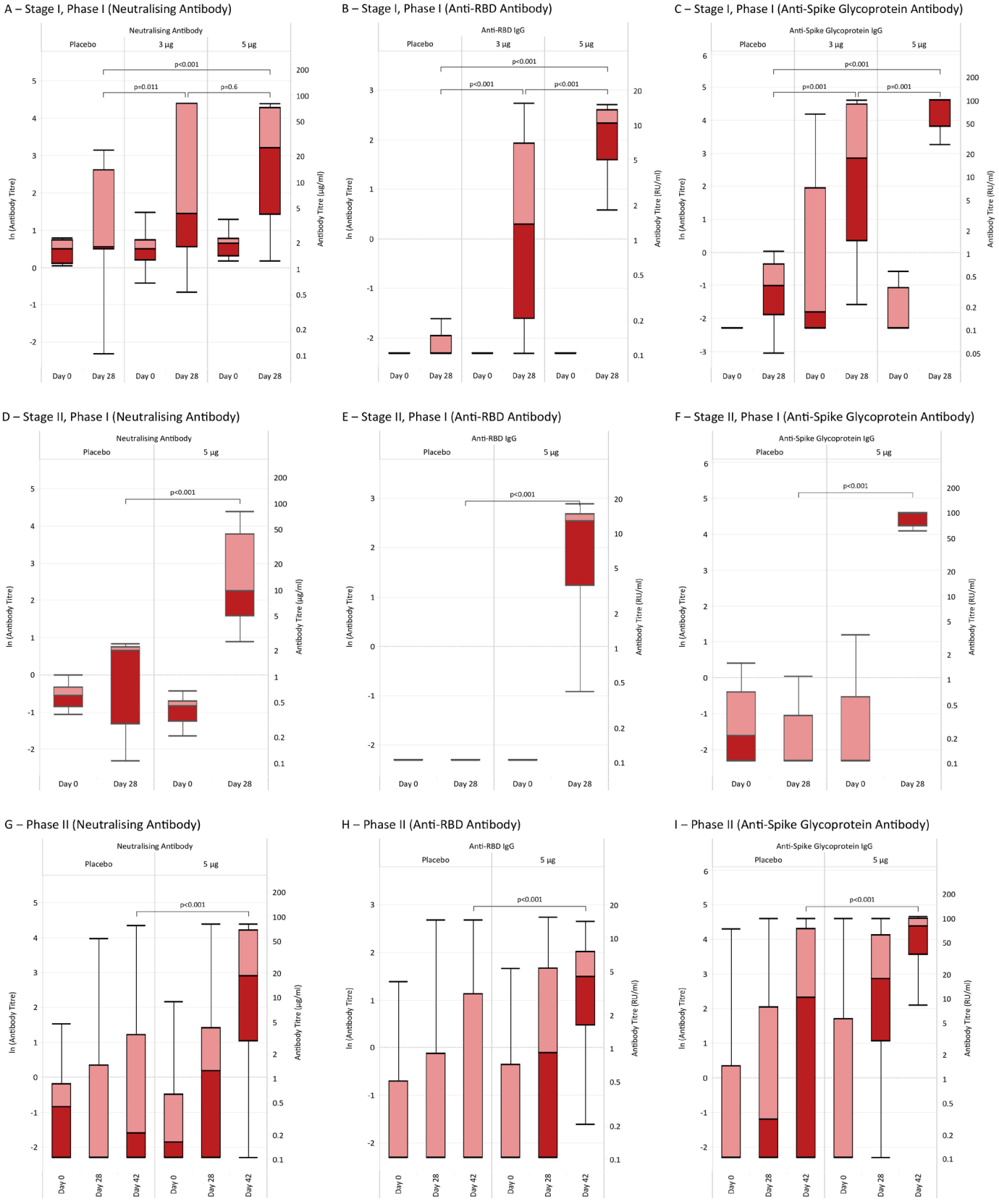

Figure 5 Anti-SARS-CoV-2 antibody titres for neutralising, anti-RBD, and anti-spike glycoprotein antibodies in stage I (A, B, C), stage II (D, E, F) and phase II (G, H, I). Box plots present second quartile in dark red and third quartile in pale red. RBD, receptor binding-domain.

Phase	Age Group	Group	Day	Sera Dilutions 1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Phase I	18-50	Placebo	Day 28	50.0%	12.5%	12.5%	12.5%	12.5%	0.0%	0.0%	0.0%	0.0%	0.0%
		3 µg	Day 28	95.8%	95.8%	83.3%	62.5%	62.5%	50.0%	45.8%	29.2%	20.8%	0.0%
		5 µg	Day 28	95.8%	95.8%	95.8%	91.7%	91.7%	91.7%	83.3%	50.0%	16.7%	0.0%
	51-75	Placebo	Day 28	75.0%	37.5%	37.5%	25.0%	25.0%	25.0%	12.5%	12.5%	0.0%	0.0%
		5 µg	Day 28	100.0%	100.0%	100.0%	95.5%	86.4%	77.3%	59.1%	18.2%	0.0%	0.0%
Phase II	18-75	Placebo	Day 28	63.2%	39.5%	18.4%	15.8%	13.2%	10.5%	10.5%	7.9%	0.0%	0.0%
			Day 42	66.7%	39.4%	18.2%	9.1%	9.1%	9.1%	6.1%	6.1%	3.0%	0.0%
		5 µg	Day 28	96.0%	85.5%	73.4%	62.9%	56.5%	54.0%	51.6%	41.9%	4.8%	0.0%
			Day 42	100.0%	96.3%	93.8%	92.5%	88.8%	82.5%	73.8%	55.0%	35.0%	0.0%

Figure 6 Proportion of serially diluted plasma samples, which neutralised wild-type SARS-CoV-2 virus in conventional virus neutralisation test among participants of phase I and phase II.

9/56 (16.1%) participants in the first injection of the placebo and 10/56 (17.9%) participants in the second injection of the placebo (online supplemental appendix 2, table S6).

There were no reports of AESI defined for COVID-19 vaccines in phase II clinical trial. No medical intervention was required after vaccination, except for the administration of paracetamol. One AE was classified as serious; one participant passed away on day 24 after receiving one injection (5 µg vaccine). The cause of death was documented as suicide via cyanide toxicity after an investigation by forensic medicine specialists, Iranian Legal Medicine Organisation and DSMB, and was considered unrelated.

Immunogenicity

Titres (GMTs) of all anti-SARS-CoV-2 antibodies, including neutralising, anti-RBD and anti-spike antibodies, increased after the first injection and on day 28 reached 1.3 (95% CI 0.9 to 1.7), 1.0 (95% CI 0.8 to 1.2) and 8.8 (95% CI 6.4 to 12.1), respectively. Following the second injection, the GMT of the neutralising, anti-RBD and anti-spike glycoprotein antibodies continued the sharp increase and on day 42 reached 11.4 (95% CI 8.7 to 15.0), 2.9 (95% CI 2.4 to 3.5) and 37.8 (95% CI 29.6 to 48.3), respectively. In contrast, the GMT of corresponding antibodies in the placebo group was 0.7 (95% CI 0.4 to 1.3), 0.4 (95% CI 0.3 to 0.7) and 3.8 (95% CI 1.7 to 8.4) on day 42. The seroconversion rates of all anti-SARS-CoV-2 antibodies reached 75% on day 42; with the most increase for neutralising antibodies with the rate of 82.8% (95% CI 77.0% to 87.6%) vs 25.5% (95% CI 14.7% to 39.0%) in the control group (table 6, figure 5G–I). The seroconversion rate of Euroimmun anti-spike glycoprotein was 83.3% (95% CI 77.5% to 88.1%) on day 42

Table 5 Baseline characteristics of participants in phase II

Characteristics	18–50 years		51–75 years	
	Placebo (N=40)	Intervention (N=160)	Placebo (N=16)	Intervention (N=64)
Sex (N, %)				
Female	15 (37.5)	50 (31.2)	7 (43.7)	30 (46.9)
Male	25 (62.5)	110 (68.8)	9 (56.3)	34 (53.1)
Age (mean-SD)	34.2 (8.7)	35.6 (7.8)	55.8 (3.0)	58.6 (6.2)
Underlying conditions (N,%)				
Chronic hypertension	1 (2.5)	8 (5.0)	1 (6.3)	15 (23.4)
Diabetes mellitus	0 (0.0)	0 (0.0)	0 (0.0)	6 (9.4)
Hyperlipidaemia	0 (0.0)	0 (0.0)	3 (18.8)	10 (15.6)
Hypothyroidism	1 (2.5)	7 (4.4)	0 (0.0)	9 (14.1)
Baseline vital signs (mean-SD)				
Body temperature (°C)	36.6 (0.3)	36.6 (0.3)	36.7 (0.2)	36.7 (0.2)
Respiratory rate (per minute)	15.0 (0.8)	15.0 (0.9)	15.1 (0.7)	15.0 (0.5)
Heart rate (beats per minute)	82.3 (11.2)	83.5 (8.3)	78.8 (7.0)	79.1 (9.8)
Systolic blood pressure (mm Hg)	122.1 (8.8)	122.1 (12.2)	122.9 (11.0)	126.7 (9.1)
Diastolic blood pressure (mm Hg)	79.2 (7.7)	79.1 (7.0)	76.6 (6.0)	77.5 (7.1)

Table 6 Geometric mean titres, geometric mean ratios, and seroconversion rates of neutralising, anti-receptor-binding domain, and anti-spike glycoprotein antibodies at different time points in phase II

	Geometric mean titre† (95% CI)		Geometric mean ratio (95% CI)	Seroconversion rate* (%) (95% CI)	
Antibody	**5 µg**	**Placebo**	**5 µg**	**5 µg**	**Placebo**
Neutralising antibody					
Day 0	0.27 (0.23 to 0.33)	0.39 (0.26 to 0.58)	0.69 (0.46 to 1.04)	N/A	N/A
Day 28	1.27 (0.94 to 1.73)	0.37 (0.23 to 0.59)	3.42 (1.80 to 6.50)	50.24 (42.26 to 56.21)	12.73 (5.27 to 24.48)
Day 42	11.44 (8.72 to 15.01)	0.67 (0.36 to 1.25)	17.05 (9.21 to 31.57)	82.78 (76.96 to 87.63)	25.45 (14.67 to 39.00)
Anti-receptor binding domain IgG					
Day 0	0.22 (0.19 to 0.25)	0.22 (0.16 to 0.32)	0.98 (0.70 to 1.39)	N/A	N/A
Day 28	0.96 (0.75 to 1.23)	0.29 (0.18 to 0.46)	3.31 (1.93 to 5.66)	51.20 (44.21 to 58.15)	20.00 (10.43 to 32.97)
Day 42	2.87 (2.39 to 3.46)	0.41 (0.25 to 0.68)	6.94 (4.49 to 10.74)	77.03 (70.73 to 82.55)	29.09 (17.63 to 42.90)
Anti-spike glycoprotein IgG					
Day 0	0.57 (0.42 to 0.77)	0.48 (0.26 to 0.88)	1.20 (0.62 to 2.31)	N/A	N/A
Day 28	8.78 (6.37 to 12.11)	0.99 (0.50 to 1.97)	8.87 (4.34 to 18.13)	68.42 (61.65 to 74.66)	23.64 (13.23 to 37.02)
Day 42	37.80 (29.61 to 48.25)	3.83 (1.74 to 8.43)	9.88 (5.32 to 18.36)	79.90 (73.82 to 85.12)	45.45 (31.97 to 59.45)

Results reported at baseline (day 0), 4 weeks after the first vaccination (day 28), and 2 weeks after the second vaccination (day 42) for 5 µg and placebo groups. In phase II, 11 participants in the 5 µg group were excluded from the study due to positive RT-PCR for COVID-19 after first injection (N=9), death due to suicide via cyanide toxicity (N=1) and coadministration of another COVID-19 vaccine platform without prior notice (N=1).
*Defined as a postvaccination titre that was at least four-fold higher than the baseline titre.
†Geometeric mean titres for neutralising antibody is reported in µg/mL—anti-receptor binding domain IgG in RU/mL—and anti-spike glycoprotein IgG in RU/mL.
NA, not applicable; RT-PCR, reverse transcription PCR.

in the 5 µg group (online supplemental appendix 2 table S5). In cVNT, the sera at 1/64 times dilution of some 82.0% of vaccinated participants with 5 µg BIV1-CovIran neutralised SARS-CoV-2 on day 42. In contrast, less than 10% of the participants' sera at the same dilution neutralised the virus in the placebo group (figure 6).

DISCUSSION

This study presents the findings from phase I and II clinical trials of BIV1-CovIran, an inactivated whole virus particle vaccine for SARS-CoV-2, adjuvanted with aluminium hydroxide. In either phase of the study, no AESI occurred, nor were any clinically significant abnormalities in laboratory values seen. Thus, the vaccine was well tolerated in both 3 µg and 5 µg dosages. Follow-up of phase I participants showed that neutralising antibody titres increased in all groups, though the antibody level rise was more prominent in the group receiving 5 µg vaccine dosage. Moreover, vaccine injection induced significant seroconversion in the intervention group. The sera at 1/64 times dilution of 92%, 77% and 82% of vaccinated participants could neutralise SARS-CoV-2 in phase I-stage I, phase I-stage II and phase II clinical trials, respectively. The ethical committee did not allow a phase I clinical trial to be conducted among people aged >50 without evidence of safety among younger age groups. Thus, phase I clinical trial was conducted in two stages, with the first stage focusing on people aged 18–50. Once the preliminary evidence for the vaccine's safety was provided for the ethical committee, permission for the conduction of stage II was granted. Moreover, participants aged 51–75 years were not recruited in phase II, until safety results from that age group in phase I were available.

The most common AE in both phases was injection site pain. No vaccine-related serious or life-threatening AEs were reported. Moreover, there were no clinically significant differences in safety among the study groups. The vaccine and the placebo both contained the same aluminium hydroxide adjuvant, a common adverse effect of which could be injection site pain and tenderness.[45]

The incidence of local and systemic AEs after both vaccine doses in this study was similar to that of other inactivated SARS-CoV-2 vaccines,[24 26] and lower than that of other SARS-CoV-2 vaccine platforms at the time of study.[46–49] Nevertheless, further studies are required to compare the short-term and long-term safety across all SARS-CoV-2 vaccine platforms.

BIV1-CovIran induced the production of neutralising antibodies, and the seroconversion rates of vaccine recipients ranged from 70.8% to 100% in phase I and phase II. The seroconversion rates were comparable to reports from phase I and phase II clinical trials of other SARS-CoV-2 vaccines: BBV152,[26] BBIBP-CorV,[24] mRNA-1273[47] and Ad26 and rAd5.[50]

In phase I, the immune response induced by the 5 µg dosage among participants aged 18–50 years was more prominent and persistent than the 3 µg dosage. Thus, the 5 µg dosage was selected for stage II of phase I and phase II clinical trials. In phase I, the two vaccine doses were administered on days 0 and 14. Nevertheless, the days 0 and 28 vaccination schedule was planned for phase II, based on the promising results of the vaccines with the same platform,[24 25 51] which would make the schedule suitable for potential routine use. The immune persistence of the two schedules needs to be further evaluated in future studies.

The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing.[13] In phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28% and 25.5% on day 42. Moreover, 10% of the participants' sera in the placebo group in 64-times dilution deactivated the wild-type virus. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants have been exposed to the virus, which could result in seroconversion, reported earlier as well.[26] Future studies need to assess whether the antibody response among vaccinated participants could be inflated due to subclinical COVID-19 infection.

To assess vaccine efficacy and further evaluate the safety outcomes, a phase III clinical trial is being conducted since 16 June 2021 with the participation of 20 000 volunteers aged 18–75 years in six cities in Iran. After random assignment to 5 µg or placebo group, participants received the intervention twice on days 0 and 28. All participants are followed up for efficacy or any AEs. Moreover, a subsample including 400 participants is being followed for immunogenicity. The phase III clinical trial protocol summary is available elsewhere.[20]

Based on the follow-up data, BIV1-CovIran has the potential to provide humoral immune responses. Considering the catastrophic toll of COVID-19 in Iran, the public rollout of a safe domestic COVID-19 vaccine could be a valuable solution. This study assessed the antibody response by determining the geometric mean titres and the seroconversion rates of neutralising, anti-RBD and anti-spike-glycoprotein antibodies in both phases. Moreover, a cVNT was performed to evaluate the levels of functional antibodies raised against SARS-CoV-2 in phase I. Nevertheless, cellular immunity induced by vaccination was not assessed in the study. The pharmaceutical company has also submitted the clinical trial documentation to WHO for emergency use consideration. In the early stages of the study, only diagnostic kits were accessible for COVID-19 in Iran, and research authorised kits were not commercially available. Thus, based on the current kits in the recruiting phase of the study, all eligible participants needed to be negative for COVID-19 RT-PCR as well as anti-nucleocapsid IgM and IgG. After proper COVID-19 neutralising antibody detection kits were available, all the collected samples at the baseline were checked, and some samples became positive. Subsequently, a sensitivity analysis was conducted, and all

participants with positive samples for neutralising antibodies in the baseline were excluded. The results of the sensitivity analysis are presented in online supplemental appendix 2 tables S7–S9.

Conclusions

Administration of the two shots of 5 µg dose BIV1-CovIran vaccine with a 28-day interval has demonstrated the potential to enhance the immunity of vaccine recipients against SARS-CoV-2 with no serious vaccine-related SAEs. These results support further evaluation of this inactivated whole virus particle vaccine in phase III.

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Contributors Conceptualisation: HH, MM, MS and PT; Data curation: HH, MAP, KH and MN; Formal Analysis: EG, NA, M-RM, MA-K, and S-HG; Funding acquisition: HH; Investigation: HH; Methodology: HH and MAP; Project administration: HH, MM, MS, PT, KH, MN and AAA; Resources: HH, MM, MS, PT and AAA; Supervision: HH, MM, MS, PT and AAA; Validation: BL; Visualisation: EG, NA, M-RM, MA-K and S-HG.; Writing-original draft: MA-K, S-HG, and NR; Writing-review and editing: HH, MM, MS, PT, AAA, BL, KH, MN, EG, NA, M-RM, NR, SSM, MA-K and S-HG; Guarantor: HH.

Funding The project was funded by Shifa Pharmed Industrial Group. Shifa Pharmed is a part of Barkat Pharmaceutical Group, which belongs to EIKO/Setad.

Competing interests HH: as manager of the Clinical Trial Center (CTC), an academic CRO affiliated with Tehran University of Medical Sciences, Tehran, Iran, I was responsible for the conduct and monitoring of clinical trials. I was a non-voting member of the Data Safety Monitoring Board, as mandated by the national regulatory authority. MM: a research contract between Shifapharmed (sponsor) and Iranian Research Center for HIV/AIDS (IRCHA) for supervising all clinical trial activities of phases one and two has been signed for 1,575 million Iranian rials, which has been deposited into the account number of this center at Tehran University of Medical Sciences. My position at the time was director of this center; as such, the payment appears to be transferred to my name in Shifa's financial statements.PT: I had the role of principal investigator in another vaccine project (Spikogen).

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The clinical trial protocols were approved by the National Research Ethics Committee (IR.NREC.1399.003 and IR.NREC.1399.007 for phase I, and IR.NREC.1399.008 for phase II).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Deidentified, individual participant data will be made available when the trial is complete, on requests directed to the corresponding author; after the approval of a proposal, data can be shared through a secure online platform.

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Correction: *Safety and immunogenicity of an inactivated virus particle vaccine for SARS-CoV-2, BIV1-CovIran: findings from double-blind, randomised, placebo-controlled, phase I and II clinical trials among healthy adults*

Mohraz M, Salehi M, Tabarsi P, *et al*. Safety and immunogenicity of an inactivated virus particle vaccine for SARS-CoV-2, BIV1-CovIran: findings from double-blind, randomised, placebo-controlled, phase I and II clinical trials among healthy adults. *BMJ Open* 2022;12:e056872. doi: 10.1136/bmjopen-2021-056872

In the corrected version of the article, the competing interest statements for authors Hamed Hosseini, Minoo Mohraz, and Payam Tabaris have been changed. The original article indicated they had no competing interests. It now states the following:

HH: as manager of the Clinical Trial Center (CTC), an academic CRO affiliated with Tehran University of Medical Sciences, Tehran, Iran, I was responsible for the conduct and monitoring of clinical trials. I was a non-voting member of the Data Safety Monitoring Board, as mandated by the national regulatory authority.

MM: a research contract between Shifapharmed (sponsor) and Iranian Research Centre for HIV/AIDS (IRCHA) for supervising all clinical trial activities of phases one and two has been signed for 1575 million Iranian rials, which has been deposited into the account number of this centre at Tehran University of Medical Sciences. My position at the time was director of this centre; as such, the payment appears to be transferred to my name in Shifa's financial statements.

PT: I had the role of principal investigator in another vaccine project (Spikogen).

The funding statement was corrected to include the organisations of which Shifa is a part. The funding statement previously stated, "The project was funded by Shifa Pharmed Industrial Group". It now states, "The project was funded by Shifa Pharmed Industrial Group. Shifa Pharmed is a part of Barkat Pharmaceutical Group, which belongs to EIKO/Setad".

A sentence of the conclusion was overstated. It said, "Administration of the two shots of 5µg dose BIV1-CovIran vaccine with a 28-day interval would enhance the immunity of all vaccine recipients against SARS-CoV-2 with no vaccine-related SAEs. The conclusion now says, "Administration of the two shots of 5µg dose BIV1-CovIran vaccine with a 28-day interval has demonstrated the potential to enhance the immunity of vaccine recipients against SARS-CoV-2 with no serious vaccine-related SAEs". The remainder of the concluding remarks are accurate.

A tracked changes online supplemental file 1 is appended to the paper for transparency.

BMJ Open 2022;**12**:e056872corr1. doi:10.1136/bmjopen-2021-056872corr1

Supplemental appendix 1

STUDY PROTOCOL

Double-Blind, Randomized, Placebo-Controlled, Phase I/II Clinical Trials to Assess Safety, Tolerability, and Immunogenicity of an Inactivated Virus Particle Anti-SARS-CoV-2 Vaccine Candidate, BIV1-CovIran, among Healthy Individuals

SHIFA
Shifa PharMed Industrial Group Co.
Biotech Production Facility

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SHIFA
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1. Protocol Summary

Synopsis

Short title

A Phase I/II Clinical Trials to Assess Safety, Tolerability, and Immunogenicity of an Anti-SARS-CoV-2 Vaccine Candidate, BIV1-CovIran, among Healthy Individuals

Rationale

Coronavirus Disease-2019 (COVID-19) pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2), still takes a toll on healthcare systems in Iran. Invaluable endeavours have been made to restrain the SARS-CoV-2 transmission chain by vaccines, and in late 2020, the first anti-SARS-CoV-2 vaccine received emergency approval for public rollout.

Notwithstanding such impressive achievements, the production and distribution of billions of vaccine doses around the globe remain challenging. Therefore, prompt administration of home-grown vaccines would be valuable for pandemic containing.

Virus inactivation was one of the first-ever, safe, and established vaccine production methods, and China and India have manufactured anti-SARS-CoV-2 vaccines so far. Iran's previous experiences in inactivated vaccine production technology have led to developing an inactivated whole virus particle vaccine for SARS-CoV-2, BIV1-CovIran. In-vivo immunogenicity and the protection of the BIV1-CovIran vaccine have been recently reported.

Objectives and Endpoints

Stage I-Phase I

Objectives	Endpoints

Primary	
Evaluation of the safety and tolerability of BIV1-CovIran vaccine candidate in two different doses (3 & 5µg antigen single human dose (SHD) of 0.5 mL) administered intramuscularly among participants aged 18 to 50 years.	1) The occurrence of any immediate reactions (local and systemic) after each administration (days 0, 14; up to 30 minutes) 2) The occurrence of any local reactions at the injection site after each administration (including pain, itching, swelling, inflammation, redness, skin rash, and irritation up to 7 days after administration (day 0 to 7 and day 14 to 21) 3) The occurrence of any systemic reactions after each administration (including fever, headaches or chills, diarrhoea, nausea, fatigue, myalgia, arthralgia, shortness of breathing, and other allergic reactions) up to 7 days after administration (day 0 to 7 and day 14 to 21) 4) The occurrence of any solicited adverse events (AEs) up to 7 days after administration (day 0 to 7 and day 14 to 21) 5) The occurrence of any Serious Adverse Events (SAEs) after each administration (day 0 to 7 and day 14 to 21)

Secondary	
Assessment of the tolerability and immunogenicity responses elicited by BIV1-CovIran vaccine candidate in two different doses (3 & 5µg antigen single human dose (SHD) of 0.5 mL) among healthy 18-50-year participants after each administration	1) The occurrence of any systemic reactions from day 8 to day 28 after each administration 2) The occurrence of any AEs from day 8 to day 28 after administration 3) The occurrence of any SAEs from day 8 to day 28 after administration 4) The assessment of immunogenicity in terms of Geometric Mean Titres (GMT) and Geometric Mean Ratios (GMRs) of anti-spike, anti-receptor binding domain (RBD), and neutralising antibodies detected by Enzyme-Linked Immunosorbent Assay (ELISA) 5) The four-fold seroconversion rate of neutralising, anti-RBD, and anti-spike antibodies detected by ELISA 6) The immunogenicity assessment in terms of increase in neutralising antibodies against SARS-CoV-2 compared to baseline in all treatment groups at days 14, 21, and 28 detected by ELISA 7) The assessment of T-Cell lymphocyte subset count and cytokines

	8) SARS-CoV-2 infection occurrence 9) Finding the appropriate dosage of vaccine optimum for immunogenicity and safety

Stage II-Phase I

Objectives	Endpoints
Primary	
Evaluation of the safety and tolerability of BIV1-CovIran vaccine (selected dosage of antigen SHD of 0.5 mL) administered intramuscularly among participants aged 51 to 75 years	1) The occurrence of any immediate reactions (local and systemic) after each administration (days 0, 14; up to 30 minutes) 2) The occurrence of any local reactions at the injection site after each administration (including pain, itching, swelling, inflammation, redness, skin rash, and irritation up to 7 days after administration (day 0 to 7 and day 14 to 21) 3) The occurrence of any systemic reactions after each administration (including fever, headaches or chills, diarrhoea, nausea, fatigue, myalgia, arthralgia, shortness of breathing, and other allergic reactions) up to 7 days after administration (day 0 to 7 and day 14 to 21)

	4) The occurrence of any solicited adverse events (AEs) up to 7 days after administration (day 0 to 7 and day 14 to 21) 5) The occurrence of any Severe Adverse Events (SAEs) after each administration (day 0 to 7 and day 14 to 21)
Secondary	
Assessment of tolerability of BIV1-CovIran vaccine candidate among healthy 51-75-year participants after each administration	1) The occurrence of any systemic reactions from day 8 to day 28 after each administration 2) The occurrence of any AEs from day 8 to day 28 after administration 3) The occurrence of any SAEs from day 8 to day 28 after administration 4) The assessment of immunogenicity in terms of GMTs and GMRs of anti-spike, anti-RBD, and neutralising antibodies detected by ELISA 5) The four-fold seroconversion rate of neutralising, anti-RBD, and anti-spike antibodies detected by ELISA 6) The immunogenicity assessment in terms of increase in neutralising antibodies against SARS-CoV-2 compared to baseline in all treatment

	groups at days 14, 21, and 28 detected by ELISA 7) The assessment of T-Cell lymphocyte subset count and cytokines 8) SARS-CoV-2 infection occurrence

Phase II:

Objectives	Endpoints
Primary	
Assessment of immunogenicity responses elicited by BIV1-CovIran vaccine candidate (selected dosage of antigen SHD of 0.5 mL) among 18-75-year participants after each administration	1) The assessment of serum IgG antibody levels specific for the SARS-CoV-2 protein antigens (neutralising, anti-RBD, and anti-spike glycoprotein antibodies) as detected by ELISA at days 14, 28,42, 56, 70, 118, 208, and day 388. Derived/calculated endpoints based on these data will include geometric mean ELISA units, geometric mean fold rise, and seroconversion rate (proportion of

	participants with ≥4-fold rises in ELISA units). 2) The assessment of T-Cell lymphocyte subset count and cytokines
Secondary	
Evaluation of the safety and tolerability of BIV1-CovIran vaccine candidate administered intramuscularly among participants aged 18 to 75 years	1) The occurrence of any immediate reactions after each administration (days 0, 28; up to 30 minutes) 2) The occurrence of any solicited AEs at the injection site after each administration (including pain, itching, swelling, inflammation, redness, skin rash, and irritation, day 0 to 7 and day 28 to 42) 3) The occurrence of any solicited systemic AEs after each inoculation (including fever, headaches or chills, diarrhoea, nausea, fatigue, myalgia, arthralgia, shortness of breathing, and other allergic

	reactions, day 0 to 7 and day 28 to 42) 4) The occurrence of any SAEs after each administration (day 0 to 7 and day 28 to 42) 5) SARS-CoV-2 infection occurrence

Overall Design

This randomised, placebo-controlled, parallel-designed, double-blind (participants and outcome assessor) clinical trial will be conducted in accordance with the declaration of Helsinki, Good Clinical Practice (GCP), and Iran GCP as a local regulation. The study protocol will be fully explained to volunteers at screening, and all participants will provide written informed consent before enrollment. An independent data and safety monitoring board (DSMB) will periodically evaluate the data and advise the outcome assessors about the clinical trials' continuation, suspension, or early termination.

Phase I and II will be conducted to evaluate the safety, tolerability, and immunogenicity of the inactivated whole virus particle vaccine candidate, BIV1-CovIran. The participant, outcome assessor, study coordinator, and other site staff will be blinded. Only the Contract Research Organization (CRO), who was responsible for labelling and data administration, will be unblinded at the study site.

This study is considered in three parts: 1) Stage I of Phase I among 18-to-50-year-old participants; 2) Stage II of Phase I among 51-to-75-year-old participants; and 3) Phase II conducted among participants aged 18 to 75.

This study evaluates the safety and immunogenicity of BIV1-CovIran vaccine candidates. The study will be conducted among healthy individuals in Stage I and Stage II of Phase I and Phase II. It is worth mentioning that all patients with the mild controlled disease will be recruited in the study, similar to other healthy individuals in Phase II. In addition, the appropriate vaccine dosage and schedule are evaluated in Phase I. The vaccine schedule in this study is as follows:

Stage I-Phase I	**Stage II- Phase I**	**Phase II**
Dosage		
3µg, 5µg and placebo with the randomization block of 3:3:1	Selected dosage of vaccine candidate and placebo with the randomization block of 3:1	Selected dosage of vaccine candidate and placebo with the randomization block of 4:1
Interval		
The 14-day interval among two shots of vaccine candidate or placebo	The 14-day interval among two shots of vaccine candidate or placebo	The 28-day interval among two shots of vaccine candidate or placebo

Phase I study will consist of a screening period (days -7 to -1); administration days (days 0 and 14); daily visits during 7 days after each administration, day 21 (± 3 days), 28 (±3 days), 42 (± 3 days), 90 (± 7 days), 180 (±7 days); and 360 (±7 days). All participants will be stayed at the trial site and will be closely monitored for seven days after each administration in Stage I and 48 hours after each administration in Stage II. Phase II study will consist of a screening period (days -7 to -1); administration days (days 0 and 28); daily visits during 7 days after each administration, day 42 (± 3 days), 56 (±3 days), 70 (± 3 days), 90 (± 7 days), 180 (±7 days); and 360 (±7 days). The comprehensive report of all participants will be collected during 28 days of trial in both Phase I and II and be reported to Iran

Food and Drug Administration (IFDA) and National Ethics Committee (NEC). Participants will be followed up for one year in various intervals, and the results will be reported to IFDA and NEC.

Before starting the screening, all trial aims and events will be thoroughly explained for volunteers, and then informed consent will be provided. Each eligible volunteer will be asked to provide written consent to use samples for future testing or assay development specific to SARS-CoV-2 (or related variants) in the screening visit. Participants will be randomly allocated to the arms of the study based on the randomization master sheet and the specific design of each phase.

The primary study site, where enrollment, injections, participant monitoring, and follow-up visits will take place, will be Eram Hotel, Tehran, Iran. If necessary, Imam Khomeini Hospital Complex will provide medical attention and hospitalisation.

Number of Participants

In Stage I, participants will be divided into intervention and placebo groups with the ratio of 3:3:1. Randomisation will be conducted in two stages. Initially, 14 participants will be randomised to receive the 3µg dosage of the vaccine or placebo (12 versus 2). Participants will be monitored for seven days after injection and followed by a DSMB meeting to approve the vaccine candidate's safety and authorise further proceeding. The remaining 42 individuals will be randomised to the 3µg, 5µg, and placebo arms. A computer will generate the randomisation sequence in a block size of seven. Two types of randomisation blocks will be used, corresponding to the two randomisation steps. The first two blocks will be used to allocate six participants to the 3µg vaccine group and one to the placebo group. The remaining six blocks will be randomised with an allocation ratio of 2:4:1, in which participants will be assigned to three study

groups: 3µg of the vaccine candidate, 5µg of the vaccine candidate, or placebo, respectively

In Stage II of Phase I, volunteers aged 51-75 years will be enrolled to randomly receive the selected dosage of the vaccine candidate (based on interim findings of Stage I) or placebo on days 0 and 14. The randomisation sequence will be computer-generated in permuted blocks of size four with an allocation ratio of 3:1. An interactive web response system will perform all random allocation processes. The total number of participants in Stage I and Stage II is estimated to be 56 (24 for 3µg, 24 for 5µg and 8 for placebo group) and 32 (24 for selected dosage of vaccine candidate and 8 for the placebo group).

During Phase II, 280 participants will be allocated to the selected vaccine candidate dosage and placebo groups. The intervention to placebo ratio will be 4:1 with the randomization block of five (200 for selected vaccine candidate and 80 for placebo).

Study Duration and Settings

The primary study site is Eram Hotel, Tehran, Iran, where enrollment, injections, participant monitoring, and follow-up visits will occur. Participants are expected to participate for up to a maximum of approximately 12 months for each stage in Phase I and Phase II.

Data Monitoring Committee or Other Independent Oversight Committee

The CRO and DSMB will periodically evaluate the data and advise the outcome assessors about continuation, suspension, or early termination of the clinical trials.

Statistical Methods

Sample size

The sample size will not be determined based on the statistical power calculation. The sample size is considered sufficient to evaluate the objectives of the study. In Stage I, assuming that there are no severe AEs (grade 4) or suspected unexpected serious adverse reaction (SUSAR) related to the experimental vaccine and the occurrence of grade 3 AEs will be less than 15% among participants, it is anticipated to have trial sample size between 20-30 volunteers. Twenty-four participants will be allocated in each 3µg and 5µg group, and 8 participants in the placebo control group (56 volunteers). Similarly, in Stage II, 24 participants will receive a selected dosage of vaccine candidate and 8 participants the placebo.

Phase II will include 200 participants aged 18 to 50 years (160 participants in the selected dosage of vaccine candidate group, 40 participants in the placebo group) and 80 volunteers in the age range of 51-75 years (64 participants in the selected dosage of vaccine candidate group, 16 participants in the placebo group who will join this study after confirmation of Stage II interim findings.

All data collected from the Phase I and Phase II studies will be analyzed based on three analysis populations: Safety population, Intention-To-Treat (ITT) population, and population per-protocol set (PPS).

1) **Safety population:** Participants enrolled in the study will receive at least one dose of the study drug.
2) **ITT population**: Participants who have at least one antibody titer measurement following the administration.
3) **PPS population**: Participants who will not have significant deviations from study protocol basics.

Safety analysis

The safety outcome will be the incidence of any AEs after injections. Solicited AEs will be defined as any events which would occur from day zero to day seven

after each administration. Unsolicited AEs will be defined as any AEs occurring from day eight to day 28 after each injection.

Numbers and percentages of subjects with solicited local and systemic AEs (based on the Food and Drug Administration (FDA) toxicity grading scale) through 7 days after each vaccination will be summarized by treatment group and the maximum toxicity grade over 7 days after each vaccination. After each vaccination, the duration of solicited local and systemic AEs will be presented individually.

Unsolicited AEs will be coded by preferred term (PT) and system organ class (SOC) using the latest version of MedDRA and summarized by the treatment group. AEs related to the study vaccine will be defined for those considered as "certain", "probable", or "possible" based on the World Health Organization (WHO) Causality Assessment. Grading of AEs will be based on the FDA Guidelines for Toxicity Rating in Healthy Individuals Participating in Vaccine Studies.

Adverse events through 28 days after first vaccination; and SAE, or AEs of Specific Interest (AESI) through 360 days after final vaccination will be listed separately and summarized by treatment group.

Actual values, changes from baseline (where indicated), and toxicity grading for clinical safety laboratory test results and vital sign measurements will be summarized by the treatment group at each time point using descriptive statistics.

Immunogenicity analysis

The Full analysis set population (FAS) and PPS are used in the humoral immunogenicity analyses, and all values are converted logarithmically before analysis. The following steps are carried out to evaluate humoral immunogenicity:

1) The minimum, maximum, median, and GMT (95% confidence interval) are calculated for statistical description.
2) Two independent samples t-test: two independent samples t-test or modified t-test is used to
    a. compare serum antibody GMT before each vaccination between vaccine and placebo groups;
    b. compare serum antibody GMT before each vaccination between different vaccine groups;
    c. compare serum antibody GMT after each vaccination between vaccine and placebo groups; and
    d. compare serum antibody GMT after each vaccination between vaccine groups;
3) $\chi 2$ test, or Fisher's exact test:
4) $\chi 2$ test or Fisher's exact test is used to
    a. Compare the seroconversion rate before each vaccination among vaccine and placebo groups and different dose groups.
    b. Compare the seroconversion rate between vaccine and placebo groups and different dose groups after each vaccination.

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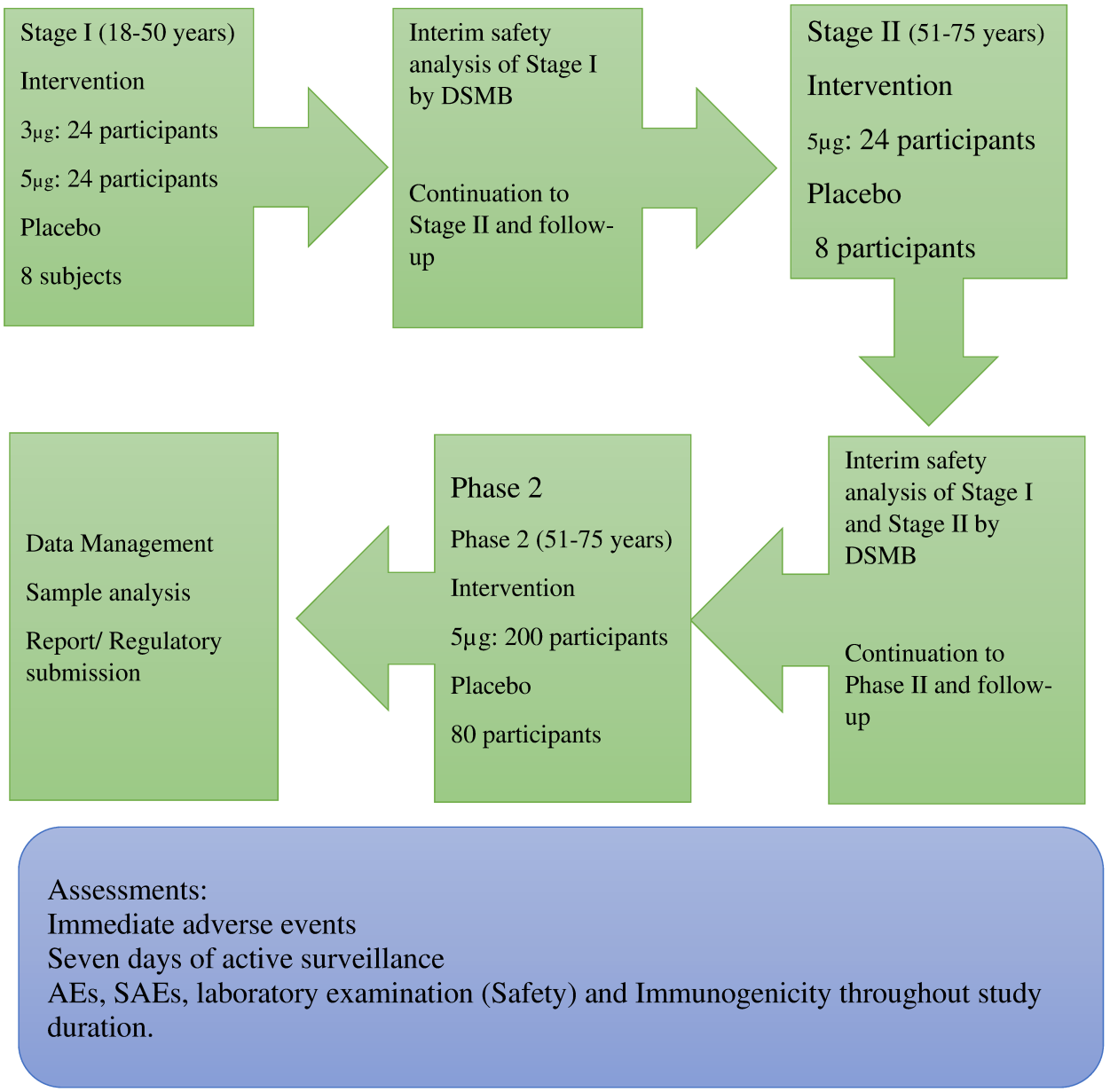
Schema



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Schedule of activity

Phase I:

Parameters	Visit 1: Screening	Visit 2 (Baseline)	Visit 3	Visit 4	Visit 5	Visit 6	The main safety report	Visit 7	Visit 8	Visit 9	The Complementary Report
Days	**-7 to -1**	**0**	**7**	**14±3**	**21±3**	**28±3**		**90±7**	**180±7**	**360±7**	
Medical History	×										
Inclusion/ exclusion criteria	×			×							
Informed consent	×										
Physical Examination	×	×	×	×	×	×					
Demography	×										
Randomization		×									
Drug history		×	×	×	×	×					
Inoculation		×		×							
RT- PCR	×										
CBC with differential test	×		×	×	×	×					
Liver function test	×		×	×	×						
Blood biochemistry test	×		×	×	×						
Urine analysis	×		×	×	×	×					
Lymphocyte subset assessment	×			×		×					
NK cell, B cell assessment	×			×		×					
Cytokine assessment	×			×		×					
Seroconversion Antibody assessment	×		×	×	×	×		×	×	×	
Neutralising Antibody assessment	×		×	×	×	×		×	×	×	
Adverse Events Assessment		×	×	×	×	×		×	×	×	

Phase II

Parameters	Visit 1: Screening	Visit 2 (Baseline)	Visit 3	Visit 4	Visit 5	Visit 6	The main safety report	Visit 7	Visit 8	Visit 9	The Complementary report
Days	**-7 to -1**	**0**	**28±3**	**42±3**	**56±3**	**70±3**		**118±7**	**208±7**	**388±7**	
Medical History	×										
Inclusion/ exclusion criteria	×		×								
Informed consent	×	×									
Physical Examination	×										
Demography	×										
Randomization		×									
Drug history		×	×	×	×	×					
Inoculation		×	×								
RT-PCR	×										
Blood sampling	×		×	×	×	×		×	×	×	
Adverse Events Assessment		×	×	×	×	×		×	×	×	

2. Introduction

Background

In December 2019, an outbreak of pneumonia occurred in Wuhan, Hubei Province, China [1]. Symptoms of disease included fever, cough, shortness of breath, and radiological changes, including patchy and diffuse infiltration. After that, a new coronavirus was identified in Wuhan from a broncho-alveolar fluid lower respiratory tract of subjects with unknown pneumonia. The coronavirus was firstly named 2019-nCoV and later as Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2). The disease named Coronavirus Disease-2019 (COVID-19). World Health Organization (WHO) defined the situation as a public health emergency, international concern, and later as a pandemic. Infection transmission was considered to be mainly through respiratory droplets and close contact with infected patients [2]. Almost everyone is susceptible to being affected, primarily the elderly and those with chronic diseases. Experts believe that safe and effective vaccines may be a potential pathway for controlling this ongoing crisis [3,4].

Inactivated vaccines have been widely used for decades and have a well-established safety profile with precise evaluation and quality control methodologies [5]. These vaccines have been used for emerging respiratory diseases and hold promise for a safe, effective, and inexpensive option against SARS-CoV-2 [5]. BIV1-CovIran is an inactivated whole virus particle vaccine that has demonstrated safety and immunogenicity in pre-clinical studies in mice, rabbits, and non-human primates [6].

Preclinical evidence

To evaluate the anti-COVID-19 inactivated vaccine candidate, animal studies were performed to evaluate the safety effects, find the number of injectable doses, and the vaccine's effectiveness in animal models of mice, rabbits, pigs, and

monkeys. The vaccine candidate challenge was also performed in mice, the results of which are summarized as follows:

Aim	Animal type	Assessments
Safety assessment	Rabbit (N=50)	Viability, weight, and disease progression
	Rabbit (N=50 randomly assigned)	Skin disorders
	Rabbits (N=4)	Pyrogenicity
	Mice (N=50)	Viability, weight, and disease progression
	Pigs (N=10)	Viability, weight, and disease progression
	Pigs (N=4)	Skin disorders
Dose finding	Rabbit (N=50)	Antibody titres
	Mice (N=50)	Antibody titres
Cytotoxicity	Vero cells of monkey kidney	Cytotoxicity
	MRC5 fibroblast cell (a human fetal lung fibroblast)	Cytotoxicity
	Primary blood cells of adult human	Cytotoxicity
Vaccine candidate challenge test	Mice (N=10)	Viability, RT-PCR, and Lung CT scan

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Stability study

Stability studies were reviewed in a laboratory model. Upon entering the clinical phase, long-term stability studies for this product will continue according to WHO guidelines.

Safety assessment

Safety tests were performed on three animal models of rabbits, mice, and pigs as follows:

1) **Safety assessment in mice**

Fifty female Balb/c mice weighing 18-16 g were prepared. Examination of the laboratory results showed no mortality and pathogenicity in any of the groups, and also, the average weight increased from 17 g to 25 g.

2) **Safety assessment in rabbits**

In this section, studies in three areas of safety, skin complications, and pyrogenicity have been reviewed. For the safety part, fifty male white Duch-Polish rabbits weighing 1800-2500 g were prepared. The animals were kept in aluminium rabbit cages in pairs at 22 ° C, 12 hours of light, 12 hours of darkness, and 55% humidity. Food and water were freely available to accustom the rabbits to the new conditions. They were kept in the laboratory for a week. Five doses of inactivated vaccine with a concentration of 5µg were administered to rabbits on days 0, 14, 21, 28, 35. Safety criteria including survival, weight, and any signs of disease in rabbits were evaluated up to 90 days after the start of injection. Examination of the laboratory results showed no mortality and pathogenicity in any of the groups, and also, the average weight increased from 1900 g to 4500 g.

For examining skin complications in rabbits, slides were randomly prepared from rabbits treated for parasitic and bacterial infections. All results show that no skin complications were observed in model animals.

Finally, for the Pyrogenicity test (fever), the non-febrile test in rabbits by the USP method showed that the total increase in body temperature of the three rabbits after the mentioned three times is below the threshold. Also, the difference between the body temperature of each animal at zero hours and the following hours was a maximum of 0.4 ° C. Therefore, it can be concluded that the candidate is not a pyrogen vaccine.

3) The safety study in pigs

As many as 14 male guinea pigs weighing 200-250 g were prepared. To test the skin sensitivity of four guinea pigs, they were randomly selected and kept in two groups. Injections in these groups were performed subcutaneously and at one time, and 14 days after injection, the induced inflammatory responses were evaluated. After two weeks, the animals were assessed, and no redness, inflammation redness, bumps, or stiffness were observed at the injection sites, and the animals were in perfect health. To evaluate the candidate vaccine's safety, seven guinea pigs were injected intraperitoneally with the vaccine, and three guinea pigs were injected intraperitoneally as control with injected sterile water. The results of this test were evaluated for 14 days. No pathological case was seen after autopsy.

Cytotoxicity

To evaluate the cytotoxicity of the vaccine candidate, three different cell types were selected, and different concentrations of the vaccine were added, and their cytotoxic effects were evaluated by MTT assay. This test showed that up to 10µg of the vaccine had no cytotoxic effects on the cell.

1) Vero cell line;
2) MRC5 fibroblast cell line, which is human fetal lung fibroblast; and
3) Primary human blood primer cells.

Immunogenicity in different animal species

To find the number of prescribed doses of antigen in the animal model in rabbits, 50 white Duch-Polish male laboratory rabbits weighing 1800-2500 g were prepared. The animals were kept in aluminium cages for rabbits in pairs at a temperature of 22 ° C, 12 hours of light, 12 hours of darkness with 55% humidity, and had free access to food and water. To accustom the rabbits to the new conditions, they were kept in the Animal Challenge Lab for a week. The aim was to evaluate the minimum number of antigen injections to establish an appropriate immune response in the animal model.

During this test, rabbits were divided into two groups of control and treatment with 20 animals. In the treatment group, five injections of antigen at the rate of 5µg in 0.5 ml per dose and in the control group 5 injections of 0.5 ml. distilled water was performed on the dates of Zero 14, 21, 28, and 35. COVID-19 antibody titers were evaluated at time zero, one week after the third injection and ten days after the fifth injection.

The results of antibody titer after receiving 3 and 5 injections indicate that the number of effective doses in rabbits is three injections. To find the number of prescribed doses of antigen in the animal model in mice, injections were performed twice on days 0 and 7 intramuscularly. Days before the injection, the second week, and the fifth week after the last injection, blood samples were taken from the retro-orbital vein of mice. Total antibody response was evaluated in experimental groups.

Humoral immune response

The vaccine candidate induced the production of specific antibodies in the experimental groups in the pre-clinical study, as investigated via two-way ANOVA followed by Tukey's post hoc test [6]. Comparing the formulated vaccine with alum adjuvant and the killed virus, it was found that the antibody titer increased in the formulated vaccine candidate group after 42 days due to

antigen storage; however, the antibody titers decreased among group that received only the killed virus after day 42. Our results also show that the antibodies have a good shelf life after 42 days of vaccination.

Challenge test

As many as 10 Balb/mice were prepared and kept in the laboratory for one week. The results indicate complete neutralization of antibodies produced by the vaccine candidate. The study of this vaccine on 4 Rhesus monkeys showed that the level of IgM against the virus after 21 days and the level of IgG after 28 days showed a significant increase.

According to the preclinical results, doses of 3 and 5µg of vaccine candidate were prepared with 95% purity and were safe and immunogenic in animal studies [6].

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3. Objectives

Stage I-Phase I

Primary objectives

- Evaluation of the safety and tolerability of BIV1-CovIran vaccine candidate in two different doses (3 & 5µg antigen single human dose (SHD) of 0.5 mL) administered intramuscularly among participants aged 18 to 50 years

Secondary objectives

- Assessment of the immunogenicity responses elicited by BIV1-CovIran vaccine candidate among healthy 18-50-year participants after each administration

Stage II-Phase I

Primary objectives

- Evaluation of the safety and tolerability of BIV1-CovIran vaccine candidate (selected dosage of antigen SHD of 0.5 mL) administered intramuscularly among participants aged 51 to 75 years

Secondary objectives

- Assessment of immunogenicity responses elicited by BIV1-CovIran vaccine candidate among healthy 51-75-year participants after each administration

Phase II

Primary objectives

- Assessment of immunogenicity responses elicited by BIV1-CovIran vaccine candidate (selected dosage of antigen SHD of 0.5 mL) among healthy 18-75-year participants after each administration

Secondary objectives

- Evaluation of the safety and tolerability of BIV1-CovIran vaccine candidate administered intramuscularly among participants aged 18 to 75 years

4. Study endpoints

Stage I-Phase I

Primary endpoints

- The occurrence of any immediate reactions (local and systemic) after each administration (days 0, 14; up to 30 minutes);
- The occurrence of any local reactions at the injection site after each administration (including pain, itching, swelling, inflammation, redness, skin rash, and irritation up to 7 days after administration (day 0 to 7 and day 14 to 21);
- The occurrence of any systemic reactions after each administration (including fever, headaches or chills, diarrhoea, nausea, fatigue, myalgia, arthralgia, shortness of breathing, and other allergic reactions) up to 7 days after administration (day 0 to 7 and day 14 to 21);
- The occurrence of any solicited adverse events (AEs) up to 7 days after administration (day 0 to 7 and day 14 to 21);
- The occurrence of any Serious Adverse Events (SAEs) after each administration (day 0 to 7 and day 14 to 21);

- Finding the appropriate dosage of vaccine optimum for immunogenicity and safety.

Secondary Endpoints

- The occurrence of any systemic reactions from day 8 to day 28 after each administration;
- The occurrence of any AEs from day 8 to day 28 after administration;
- The occurrence of any SAEs from day 8 to day 28 after administration;
- The assessment of immunogenicity in terms of Geometric Mean Titres (GMT) and Geometric Mean Ratios (GMRs) of anti-spike, anti-receptor binding domain (RBD), and neutralising antibodies detected by enzyme-linked immunosorbent assay (ELIZA);
- Four-fold seroconversion rate of neutralising, anti-RBD, and anti-spike antibodies;
- The immunogenicity assessment in terms of increased neutralising antibodies against SARS-CoV-2 compared to baseline in all treatment groups at day 14, 21, and 28;
- The assessment of T-Cell lymphocyte subset count and cytokines;
- SARS-CoV-2 infection occurrence.

Stage II-Phase I

Primary endpoints

- The occurrence of any immediate reactions (local and systemic) after each administration (days 0, 14; up to 30 minutes);
- The occurrence of any local reactions at the injection site after each administration (including pain, itching, swelling, inflammation, redness, skin rash, and irritation up to 7 days after administration (day 0 to 7 and day 14 to 21);

- The occurrence of any systemic reactions after each administration (including fever, headaches or chills, diarrhoea, nausea, fatigue, myalgia, arthralgia, shortness of breathing, and other allergic reactions) up to 7 days after administration (day 0 to 7 and day 14 to 21);
- The occurrence of any solicited AEs up to 7 days after administration (day 0 to 7 and day 14 to 21);
- The occurrence of any SAEs after each administration (day 0 to 7 and day 14 to 21).

Secondary Endpoints

- The occurrence of any systemic reactions from day 8 to day 28 after each administration;
- The occurrence of any AEs from day 8 to day 28 after administration;
- The occurrence of any SAEs from day 8 to day 28 after administration;
- The assessment of immunogenicity in terms of GMTs and GMRs of neutralising, anti-RBD and anti-spike antibodies detected by ELISA;
- The four-fold seroconversion rate of neutralising, anti-RBD and anti-spike antibodies;
- The immunogenicity assessment in terms of increased neutralising antibodies against SARS-CoV-2 compared to baseline in all treatment groups at days 14, 21, and 28;
- The assessment of T-Cell lymphocyte subset count and cytokines;
- SARS-CoV-2 infection occurrence.

Phase II

Primary endpoints

- The assessment of serum IgG antibody levels specific for the SARS-CoV-2 protein antigens (neutralising, anti-RBD, and anti-spike glycoprotein antibodies) as detected by ELISA at day 14, 28,42, 56, 70, 118, 208, and

day 388: derived/calculated endpoints based on these data will include geometric mean ELISA units, geometric mean fold rise, and seroconversion rate (proportion of participants with ≥ 4-fold rises in ELISA units);

- The assessment of T-Cell lymphocyte subset count and cytokines;
- SARS-CoV-2 infection occurrence.

Secondary endpoints

- The occurrence of any immediate reactions after each administration (days 0, 28; up to 30 minutes);
- The occurrence of any solicited AEs at the injection site after each administration (including pain, itching, swelling, inflammation, redness, skin rash, and irritation, on days 0 to 7 and days 28 to 42);
- The occurrence of any solicited systemic AEs after each inoculation (including fever, headaches or chills, diarrhoea, nausea, fatigue, myalgia, arthralgia, shortness of breathing, and other allergic reactions, day 0 to 7 and day 28 to 42);
- The occurrence of any SAEs after each administration (day 0 to 7 and day 28 to 42).

5. Study design

Overall design

This randomised, placebo-controlled, parallel-designed, double-blind (participants and outcome assessor) clinical trial will be conducted in accordance with the declaration of Helsinki, Good Clinical Practice (GCP), and Iran GCP as a local regulation. The study protocol will be fully explained to volunteers at screening, and all participants will provide written informed consent before enrollment. An independent data and safety monitoring board (DSMB) will

periodically evaluate the data and advise the outcome assessors about the clinical trials' continuation, suspension, or early termination.

Phase I and II will be conducted to evaluate the safety, tolerability, and immunogenicity of the inactivated whole virus particle vaccine candidate, BIV1-CovIran. The participant, outcome assessor, study coordinator, and other site staff will be blinded. Only the Contract Research Organization (CRO), responsible for labelling and data administration, will be unblinded at the study site.

This study is considered in three parts: 1) Stage I of Phase I among 18-to-50-year-old participants; 2) Stage II of Phase I among 51-to-75-year-old participants; and 3) Phase II conducted among participants aged 18 to 75.

This study evaluates the safety and immunogenicity of BIV1-CovIran vaccine candidates. The study will be conducted among healthy individuals in Stage I and Stage II of Phase I and Phase II. It is worth mentioning that all patients with the mild controlled disease will be recruited in the study, similar to other healthy individuals in Phase II. In addition, the appropriate vaccine dosage and schedule are evaluated in Phase I. The vaccine schedule in this study is as follows:

Stage I-Phase I	**Stage II- Phase I**	**Phase II**
Dosage		
3µg, 5µg and placebo with the randomization block of 3:3:1	Selected dosage of vaccine candidate and placebo with the randomization block of 3:1	Selected dosage of vaccine candidate and placebo with the randomization block of 4:1
Interval		
The 14-day interval among two shots of	The 14-day interval among two shots of	The 28-day interval among two shots of

vaccine candidate or placebo.	vaccine candidate or placebo.	vaccine candidate or placebo.

Phase I study will consist of a screening period (days -7 to -1); administration days (days 0 and 14); daily visits during 7 days after each administration, day 21 (± 3 days), 28 (±3 days), 42 (± 3 days), 90 (± 7 days), 180 (±7 days); and 360 (±7 days). All participants will stay at the trial site and be closely monitored for seven days after each administration in Stage I and 48 hours after each administration in Stage II. Phase II study will consist of a screening period (days -7 to -1); administration days (days 0 and 28); daily visits during 7 days after each administration, day 42 (± 3 days), 56 (±3 days), 70 (± 3 days), 90 (± 7 days), 180 (±7 days); and 360 (±7 days). The comprehensive report of all participants will be collected during 28 days of trial in both Phase I and II and be reported to Iran Food and Drug Administration (IFDA) and National Ethics Committee (NEC). Participants will be followed up for one year in various intervals, and the results will be reported to IFDA and NEC.

Before starting the screening, all trial aims and events will be thoroughly explained to volunteers, and then informed consent will be provided. Each eligible volunteer will be asked to provide written consent to use samples for future testing or assay development specific to SARS-CoV-2 (or related variants) in the screening visit. Participants will be randomly allocated to the arms of the study based on the randomization master sheet and the specific design of each phase.

The primary study site, where enrollment, injections, participant monitoring, and follow-up visits will take place, will be Eram Hotel, Tehran, Iran. If necessary, Imam Khomeini Hospital Complex will provide medical attention and hospitalisation.

Blindness

This study will be conducted as a double-blind study (participants and outcome assessor). To carry out the blinding process, vaccine candidate vials and placebo are offered precisely the same appearance, label, and unique identification code, guaranteeing the participants, researchers, and outcome assessors' blindness. After the vaccine administration, the initial of the participant and the date of vaccination are written in the outer packaging box, and the label is recorded on the main sheet. Personnel checks all information before injection. During the study, all packing boxes will be archived and maintained.

Phase I

Phase I will be carried out in two stages: Stage I will include individuals aged 18-50, and Stage II will consist of individuals aged 51-75 years. In Stage I, a total of 56 volunteers aged 18 to 50 years will be randomised with an allocation ratio of 3:3:1 into three arms to receive 3µg of the vaccine (24 participants), 5µg of the vaccine (24 participants), or placebo (8 participants) on days 0 and 14. Randomisation will be conducted in two stages. Initially, 14 participants will be randomised to receive the 3µg dosage of the vaccine or placebo (12 versus 2). Participants will be monitored for seven days after injection, followed by a DSMB meeting to investigate the vaccine safety and authorise further proceeding. The remaining 42 individuals will be randomised to the 3µg, 5µg, and placebo arms. A computer will generate the randomisation sequence in a block size of seven. Two types of randomisation blocks will be used, corresponding to the two randomisation steps. The first two blocks will allocate six participants to the 3µg vaccine group and one to the placebo group. The remaining six blocks will be randomised with an allocation ratio of 2:4:1, in which participants will be assigned to three study groups: 3µg of vaccine, 5µg of vaccine, or placebo, respectively.

The preliminary results of the vaccine candidate's safety among participants aged 18-50 years will be presented to the DSMB and NEC to investigate the further progress of the study.

In Stage II of Phase I, 32 volunteers aged 51-75 years will be enrolled to randomly receive the chosen dose of the vaccine candidate (24 participants) or placebo (8 participants) on days 0 and 14. The randomisation sequence will be computer-generated in permuted blocks of size four with an allocation ratio of 3:1. An interactive web response system will perform all random allocation processes.

Phase II

In Phase II, the intervention arm will receive the selected vaccine dose based on Phase I results. On days 0 and 28, volunteers in Phase II will be stratified based on their age group—age 18-50 and 51-75 years. Participants aged 51-75 years will not be recruited in Phase II, until safety results from that age group in Phase I are available. Overall, 280 participants (200 aged 18-50 years and 80 aged 51-75 years) will be randomised with a 4:1 ratio to receive a selected dosage of vaccine shots (224 participants) or a placebo (56 participants).

Update as part of protocol amendment: In Phase II, the vaccine schedule was modified to enhance efficacy, based on the experts' opinion after early results of Phase I, as well as the emerging evidence from other studies [7–10]. Thus, participants received the vaccine candidate/placebo on days 0 and 28.

Scientific rationale for study design

Inactivated vaccines have been widely used for decades and have a well-established safety profile with precise evaluation and quality control methodologies [5]. These vaccines have been used for emerging respiratory diseases and hold promise for a safe, effective, and inexpensive option against SARS-CoV-2 [5]. Considering Iran's successful experiences in the mass-

production of vaccines of this platform [11], efforts to make domestic inactivated vaccines against SARS-CoV-2 seemed reasonable.

Justification for dose

Based on nonclinical data of the inactivated virus particle vaccine candidate, it was expected that doses in the 3- to 5µg range would be immunogenic and induce neutralising antibodies. Based on previous clinical and nonclinical experience, it was expected that the defined doses would be well tolerated.

Update as part of protocol amendment: In Phase II, the 5µg vaccine dose was selected, based on the experts' opinion after the early results of Phase I, as well as the emerging evidence from other studies [7–10]. Thus, the intervention arm received 5µg of the vaccine on days 0 and 28.

End of study definition

A participant is considered to have completed the study if they have completed all study phases, including the last visit. The end of the study is defined as the date of the last visit of the last participant in the study.

6. Study population

Healthy adults who met the inclusion/exclusion criteria for participation in the study will be selected. All relevant medical and nonmedical conditions should be considered when deciding whether a particular participant is suitable for this protocol. Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

Inclusion criteria

Stage I, Phase I

The inclusion criteria for enrollment in Stage I of Phase I are as follows:

1) Adult women and men age 18-50 years for Stage I, 51-75 years for Stage II, and 18-75 for Phase II at screening time;
2) Healthy general condition, based on medical history, clinical laboratory results, vital sign measurements, and physical examination at screening;
3) Willingness and capability of cooperation throughout the study period;
4) The ability to fully understand the study processes, including all scheduled visits, vaccination plans, laboratory tests, lifestyle considerations, and other study procedures;
5) The capability of understanding thoroughly the contents of the informed consent ability to sign it before the study start date;
6) Willingness and allowing study researchers to access to medical records, laboratory assessments in the condition of hospitalisation due to suspicion to or approval of COVID-19;
7) Having negative pregnancy test at screening or vaccination (women only);
8) Consent to contraception use throughout the study (for both women and men); and
9) Agreement on not donating whole blood, blood products, or bone marrow from the start; date of the trial start until three months after receiving the second shot.

Stage II, Phase I

The inclusion criteria for enrollment in Stage II of Phase I are as follows:

- Adult males or females between 51 and 75 years old, inclusive, at screening;
- Healthy general condition, based on medical history, clinical laboratory results, vital sign measurements, and physical examination at screening (without a history of exacerbation of the disease and hospitalization due to it in the last three months);

- Willing and able to cooperate throughout the study period according to the study protocol;
- Can fully understand the study processes and understand the explanations of the facilitators correctly;
- Can understand the contents of the informed consent form and sign it before entering the study;
- Allowed researchers access to medical records, test results if hospitalized due to suspicion, or approval of COVID-19;
- A negative pregnancy test at screening or vaccination (women only; unless menopause volunteers);
- Is using effective methods of contraception during the study (male and female);
- Volunteers who agreed not to donate blood, blood products, or bone marrow from the start of the vaccine until three months after receiving the last dose

Phase II

The inclusion criteria for enrollment in Phase II are as follows:

- Adult males or females aged between 18 and 75 years old;
- Healthy general condition, based on medical history, clinical laboratory results, vital sign measurements, and physical examination at screening (without a history of exacerbation of the disease and hospitalization due to it in the last three months);
- Willing and able to cooperate throughout the study period according to the study protocol;
- Can fully understand the study processes and understand the explanations of the facilitators correctly;
- Can understand the contents of the informed consent form and sign it before entering the study;

- Allowed researchers access to medical records, test results if hospitalized due to suspicion, or approval of COVID-19;
- A negative pregnancy test at screening or vaccination (women only; unless menopause volunteers);
- Is using effective methods of contraception during the study (male and female);
- Volunteers who agreed not to donate blood, blood products, or bone marrow from the start of the vaccine until three months after receiving the last dose

Exclusion criteria

Stage I, Phase I

- Confirmed, suspected, or asymptomatic case of COVID-19;
- Positive Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test for COVID-19;
- Positive for COVID-19 antibody (anti-nucleocapsid IgG, IgM);
- Any history of SARS-CoV-2 infection;
- Any history of contact with a person with SARS-CoV-2 infection (positive RT-PCR test) during the last 14 days;
- Self-isolation due to COVID-19 (suspicion of exposure or suspicious symptoms);
- Presenting with fever (axillary temperature greater than 37.5 ° C or sublingual temperature greater than 38 ° C);
- Having at least two symptoms compatible with COVID-19, including dry cough, extreme tiredness, nasal congestion, rhinorrhea, sore throat, myalgia, diarrhoea, dyspnea, and shortness of breath during the 14 days before trial conduction;
- Any abnormality in biochemistry, blood and urine laboratory tests;

- Any history of severe allergy or allergic reactions to inactivated vaccine components;
- Any personal or family history of seizure, epilepsy, encephalopathy, or mental disorders;
- Any congenital malformations;
- Any history of neurologic disorders, seizure, Guillain-Barre syndrome except for childhood febrile seizure);
- Any history of growth and genetic disorders;
- Any history or current signs of malnutrition;
- Having underlying conditions including hepatorenal diseases, uncontrolled hypertension (systolic and diastolic blood pressure above 140 and 90 mmHg, respectively), morbid obesity (BMI>40), diabetes, chronic heart, kidney, liver, neurological or pulmonary severe diseases in medical examinations and according to the volunteer history (significant change in the course of treatment or hospitalization due to exacerbation of the disease in the last three months);
- History of thyroid disease or thyroidectomy, splenectomy or any organ removal;
- Presenting acute diseases or an exacerbation of chronic disease in the last seven days of screening;
- Known cases of immunodeficiency, Human Immunodeficiency Virus (HIV) infection, lymphoma, leukaemia, and any other autoimmune diseases;
- Any history of coagulopathy;
- Currently known case of tuberculosis, hepatitis B, or hepatitis C;
- Receiving immunomodulators or immunosuppressors at least 14 days in the past three months;

- Any history of the administration of live vaccines within one month before the trial start date;
- Any history of the administration of other types of vaccines 14 days before the trial start date;
- History of drug or alcohol abuse;
- Receiving immunoglobulins or blood products within three months before the trial start date;
- Receiving any other investigational drug within six months before the trial start date;
- Planning to receive any vaccination within one month after administration of vaccine candidate or placebo;
- History of severe mental disorders affecting the participation in the study;
- Pregnant or lactating women or those who intend to become pregnant during the study period;
- Having a high-risk job of being exposed to the SARS-CoV-2 virus or having a high risk of exposure according to the investigator evaluation; and
- Any other condition that makes a person inappropriate for participation based on the investigator's opinion

Stage II, Phase I

- Confirmed, suspected, or asymptomatic COVID-19 detected by RT-PCR at baseline;
- COVID-19 positive antibody (anti-N IgG, IgM);
- History of SARS-CoV-2 infection;
- History of contact with a person with SARS-CoV-2 infection (positive PCR test) during the last 14 days;
- People in the home quarantine period due to COVID-19 (suspicion of exposure or suspicious symptoms);

- Fever (axillary temperature greater than 37 ° C or sublingual temperature >38°c);
- Having at least two of the following symptoms: dry cough, extreme tiredness, nasal congestion, runny nose, sore throat, muscle aches, diarrhoea, dyspnea, and shortness of breath during the 14 days before vaccination;
- Abnormality in biochemistry, blood and urine laboratory tests
- History of severe allergy or allergic reactions to Inactivated vaccine components (aluminium);
- Personal or family history of seizure, epilepsy, encephalopathy, or mental disorders;
- Congenital malformations;
- History of neurologic disorders or seizure or Guillain-Barre syndrome (excluding childhood febrile seizure);
- Growth disorders;
- Any Genetic disorder;
- History or signs of malnutrition;
- Any hepatic/renal diseases;
- Uncontrolled hypertension (systolic BP more than 140, diastolic more than 90 mmHg);
- Diabetes complications (Uncontrolled blood sugar, known neurological or vascular complications or under medical supervision);
- Body Mass Index (BMI) > 40;
- Any malignancy;
- Acute diseases or an exacerbation of chronic disease in the last seven days of screening;
- Known case of immunodeficiency, HIV, lymphoma, leukaemia, or other autoimmune diseases

- Thyroid disease or history of thyroidectomy Splenectomy or history of any organ removal;
- History of coagulopathy;
- Is receiving Anti-TB treatment;
- Positive HBsAg;
- Positive HIV test;
- Positive HCV antibody;
- Is receiving immunomodulators or immunosuppressors at least 14 days in the past 3 months;
- Has received a live vaccine in one month or other vaccines in 14 days before inoculation;
- History of drug or alcohol abuse;
- Has received immunoglobulins or blood products in 3 months before inoculation;
- Has received any other investigational drug in 6 months before inoculation;
- Had the plan to receive any vaccination in on month after inoculation;
- History of severe mental disorders affecting the participation in the study;
- Pregnant or lactating women or those who intend to become pregnant during the study period;
- Had a high-risk job of being exposed to the SARS-CoV-2 virus or had a high risk of exposure according to the investigator evaluation;
- Any other circumstances other than those mentioned above that the researcher deems inappropriate to participate in the clinical trial

Phase II

It is worth mentioning that all patients with mild controlled disease were recruited in the study, similar to other healthy individuals in Phase II.

- Confirmed, suspected, or asymptomatic COVID-19 detected by RT-PCR at baseline;
- COVID-19 positive antibody (anti-N IgG, IgM);
- History of SARS-CoV-2 infection;
- History of contact with a person with SARS-CoV-2 infection (positive PCR test) during the last 14 days;
- Fever (axillary temperature greater than 37.5 ° C or sublingual temperature greater than 38 ° C) or at least two symptoms of dry cough, extreme tiredness, nasal congestion, runny nose, sore throat, muscle aches, diarrhoea, shortness of breath, and shortness of breath during the 14 days before vaccination (if fever persists, admission to the study may be delayed for up to 72 hours without a fever.);
- History of severe allergy or allergic reactions to inactivated vaccine components (aluminium);
- Currently known case of tuberculosis, hepatitis B, or hepatitis C;
- History of coagulopathy;
- History of splenectomy;
- Any of the uncontrolled diseases like uncontrolled blood pressure (systolic and diastolic blood pressure above 140 and 90 mm Hg, respectively), diabetes, chronic heart, kidney, liver, neurological or pulmonary severe diseases in medical examinations and according to the volunteer history (significant change in the course of treatment or hospitalization due to exacerbation of the disease in the last three months). Note: All Mild to Moderate patients with the controlled disease, like other healthy individuals, should be able to attend the study.
- Acute illness or exacerbation of chronic illness in the last seven days;
- Any malignancy, immune deficiency disease, HIV, lymphoma, leukaemia, or other autoimmune disorders;

- Receiving immunomodulatory or immunosuppressive for at least 14 consecutive days in the last three months or have a plan to receive over the next year (in the case of corticosteroids, a dose equivalent to more than 20 mg per day of prednisolone for more than seven days) during the last three months. Topical and inhaled use is not included;
- Immunosuppressants include chemotherapy drugs, drugs for the treatment of MS, inflammatory diseases and other autoimmune diseases, monoclonal and polyclonal antibodies, calcineurin inhibitors (cyclosporine, tacrolimus), interleukin inhibitors, TNF inhibitors, Corticosteroids, and immune-boosting drugs including vaccines, monoclonal antibodies, polyclonal antibodies, recombinant cytokines, levamisole and isoprinosine, thymosins, and any other medication that the researcher believes affects strengthening or suppressing the immune system;
- Receiving any live vaccines one month before inoculation or other vaccines during the last 14 days;
- A history of alcohol or drug dependency over the past 12 months that has led to medical, family, and occupational disorders;
- Has received immunoglobulins or blood products in 3 months before inoculation or have a plan to receive over the next year;
- Has received immunoglobulins or blood products in 3 months before inoculation or have a plan to receive over the next year
- Received any investigational drug in 6 months before inoculation or had the plan to receive any vaccination in one month after inoculation;
- Having a plan to participate in another clinical trial during the study period;
- History of severe mental disorders affecting the participation in the study;
- Pregnant or lactating women or those who intend to become pregnant during the study period;
- Travel history to countries abroad in the 14 days before screening;

- Any other circumstances other than those mentioned above that the researcher deems inappropriate to participate in the clinical trial.

Screen Failure

Screen failures are defined as participants who consent to participate in the clinical study but are not randomly assigned to study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the CONSORT publishing requirements and respond to regulatory authorities' queries. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE (except for volunteers not included in the randomization).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened under a different participant number.

Re-Vaccination Exclusion Criteria

Eligible volunteers recruited in the trials might be discontinued from study treatment and assessments at any time. Specific reasons for stopping them from receiving the second dose of intervention are as follows:

1) Positive pregnancy test (Beta-HCG) before the second administration
2) Presenting with fever (sublingual temperature greater than 39 ° C or axillary temperature greater than 38.5 ° C) over three days or any severe allergic reaction after the first inoculation;
3) Infection with COVID-19 (positive PCR test) between two injections;
4) Reporting any severe adverse events after first administration, associated with the vaccine candidate;
5) Receiving immunoglobulin or steroidal hormones (oral or intravenous) up to two weeks before the second administration;

Withdrawal of the participants from the study will be potentially due to:

1) Refusing to continue the study;
2) Any suspicion of immunodeficiency disorders with physical examination (the laboratory tests are not obligatory);
3) Getting pregnant before the administration of the second dose of vaccine candidate or placebo;
4) Occurrence of severe hypersensitivity reactions (severe anaphylactic or anaphylactoid, bronchospasm, and severe urticaria) to intervention during 30 minutes after administration
5) Administration of other vaccines or forbidden medicines based on trial protocol;
6) Occurrence of a serious adverse event which may convince the Principal Investigator (PI) to withdraw the participant from the study;
7) Any occurrence of an acute or chronic situation which may convince the PI to withdraw the participant from the study;
8) Any deviation from the scheduled visit times based on protocol; and
9) Participation in any other clinical trials.

All participants who met the discontinuation criteria must be considered for the immediate assessment and early termination as soon as possible after the discontinuation. The outcome assessor has to record all justifications of permanent discontinuation or dropout. In cases of study discontinuation or drop-out from the study due to the reason mentioned above, the study result will be collected, reviewed, and could be included in the final assessment.

7. Study intervention

Study vaccine

BIV1-CovIran is an inactivated whole virus particle vaccine manufactured by Shifa Pharmed Industrial Group. The SARS-CoV-2 virus was isolated from the nasopharyngeal specimen of an Iranian patient with COVID-19. The virus was

sequenced and cultured using a Vero cell manufacturing platform in a biosafety level 3 (BSL-3) facility [12]. Viral particles were inactivated with β-propiolactone. After purification, the inactivated virus particles were sterilised with filtration and formulated with Alhydrogel as adjuvant (Croda International [13]).

Further details about vaccine production are presented elsewhere [6]. The placebo solution contained the same aluminium hydroxide adjuvant. Vaccine and placebo vials were stored at 2-8°C.

Dosage form and route of administration

Stage I-Phase I:

- Arm 1: BIV1-CovIran vaccine candidate
  - Dose: 0.5 mL, 3µg
  - Route and mode of administration: Intramuscular Injection (Deltoid Muscle)
  - Dosage Schedule: Day 0 & 14
- Arm 2: BIV1-CovIran vaccine candidate
  - Dose: 0.5 mL, 5µg
  - Route and mode of administration: Intramuscular Injection (Deltoid Muscle)
  - Dosage Schedule: day 0 and 14
- Arm 3: Placebo
  - Dose: 0.5 mL
  - Route and mode of administration: Intramuscular Injection (Deltoid Muscle)
  - Dosage Schedule: day 0 and 14

Stage II-Phase I:

- Arm 1: BIV1-CovIran vaccine candidate

- Dose: 0.5 mL, selected dosage of the vaccine candidate
- Route and mode of administration: Intramuscular Injection (Deltoid Muscle)
- Dosage Schedule: day 0 and 14

- Arm 2: Placebo
  - Dose: 0.5 mL
  - Route and mode of administration: Intramuscular Injection (Deltoid Muscle)
  - Dosage Schedule: day 0 and 14

Phase II:

- Arm 1: BIV1-CovIran vaccine candidate
  - Dose: 0.5 mL, selected dosage of the vaccine candidate
  - Route and mode of administration: Intramuscular Injection (Deltoid Muscle)
  - Dosage Schedule: day 0 and 28
- Arm 2: Placebo
  - Dose: 0.5 mL
  - Route and mode of administration: Intramuscular Injection (Deltoid Muscle)
  - Dosage Schedule: day 0 and 28

Identity of investigational product

(1) Investigational vaccine candidate:

- Product Name: BIV1-CovIran vaccine candidate
- Active Ingredient: Inactivated COVID-19 Antigen
- Appearance and formulation: The vaccine is a sterile opalescent, white suspension in a vial, free from extraneous particles matter, containing 5µg of inactivated SARS-CoV-2 virus and a maximum of 500 µg of Alhydrogel. with pH: 6.00 - 8.00.

- Storage method: Store at a temperature of 2~8℃
- Shelf-life: 6 months

(2) Placebo:

- Product Name: Placebo
- Active Ingredient: Not applicable
- Appearance and formulation: a sterile opalescent, white suspension in a vial, free from extraneous particles matter, containing a maximum of 500 µg of Alhydrogel, diluted by phosphate-buffered saline with pH: 6.00 - 8.00.
- Storage method: Store at a temperature of 2~8℃
- Shelf-life: 6 months

Medications during trial participation

The drug history will be taken in every visit and transferred to case report forms (CRF). Medications should not be withheld if required for a participant's medical care. The following medications are prohibited to all study participants from the time of informed consent until the completion of the study:

1) Immunosuppressant or immune modifying medication (Azathioprine, Cyclosporin, Interferon, G-CSF, Tacrolimus, Everolimus, Sirolimus, high-dose systemic corticosteroids).

2) Immunoglobulin

3) Blood derivatives

4) Other vaccines: Unless considered medically necessary, no vaccines other than study intervention should be administered within 28 days before and 28 days after each study vaccination.

8. Study procedures

Visit 1: Screening

Phase I and Phase II: (-7 Day to -1 Day)

After obtaining informed consent, all volunteers will be screened in both phases by assessing medical history, inclusion/ exclusion criteria, and physical examination. Sociodemographic data, COVID-19 RT-PCR, anti-N IgM, IgG and neutralising antibody (IgG) for COVID-19 screening, complete blood count (CBC) with a differential test, liver function, blood biochemistry, urine analysis, lymphocyte subset, cytokine will be gathered from all eligible volunteers, and they will be enrolled.

Visit 2: Randomization and first administration

Phase I and Phase II (Day 0)

All Phase I and Phase II participants will undergo physical and general examination by medical experts. Drug history will be recorded. All participants will be randomly allocated to intervention or placebo groups based on the randomization master sheet and the specific design for safety issues. In Stage I-Phase I, Stage II-Phase I, and Phase II, the randomization ratio will be 3:3:1, 3:1, and 4:1, respectively. In visit one, a vaccine dose or placebo will be administered. Following the first dose administration, any immediate adverse events will be recorded.

Visit 3

Phase I (Day 7±3)

All participants will undergo a full medical inspection including physical examination, drug history obtaining, and thorough laboratory assessments (CBC with differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine and the evaluation of the titres of neutralising, anti-RBD and anti-spike antibodies detected by ELISA). During this visit, any reported adverse events will be recorded.

Phase II (Day 28)

After a complete physical examination, obtaining the history, and recording the drug history, the diary book of the participants will be reviewed, and all required information will be registered. In addition, a complete blood sample will be obtained for immunogenicity assessments. During this visit, the second vaccine candidate or placebo dose will be administered to participants who do not meet the Re-Vaccination Exclusion Criteria.

Visit 4

Phase I (Day 14)

All participants will undergo a thorough physical examination, and a drug history will be obtained. Blood and urine sampling will be gathered for CBC with a differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine, and the assessment of the titres of neutralising, anti-RBD and anti-spike antibodies detected by ELISA. The second vaccine dose or placebo dose will be administered, and any adverse event will be recorded. During this visit, the second vaccine candidate or placebo dose will be administered to participants who do not meet the Re-Vaccination Exclusion Criteria.

Phase II (Day 42±3)

All participants will undergo a thorough physical examination, and a drug history will be obtained. Blood and urine sampling will be gathered for CBC with a differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine, and the assessment of the titres of neutralising, anti-RBD, and anti-spike antibodies detected by ELISA. Any reported adverse events and the diary book of the participants will be reviewed, and all required information will be recorded.

Visit 5

Phase I (Day 21±3)

All participants will undergo a thorough physical examination, and a drug history will be obtained. Blood and urine sampling will be gathered for CBC with a differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine, and the assessment of the titres of neutralising, anti-RBD anti-spike antibodies detected by ELISA. Any reported adverse events and the diary book of the participants will be reviewed, and all required information will be recorded.

Phase II (Day 56±3)

A physical examination is performed during this visit, the volunteers' history is taken, and possible adverse events are recorded. A blood sample is taken from volunteers to assess the immunogenicity response. Other additional tests will be requested in cases of any abnormalities in participants' examination and history.

Visit 6

Phase I (Day 28±3)

All participants will undergo a thorough physical examination, and a drug history will be obtained. Blood and urine sampling will be gathered for CBC with a differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine, and the assessment of the titres of neutralising, anti-RBD anti-spike antibodies detected by ELISA. Any reported adverse events and the diary book of the participants will be reviewed, and all required information will be recorded.

Phase II (Day 70±7)

All participants will undergo a thorough physical examination, and a drug history will be obtained. Blood and urine sampling will be gathered for CBC with a

differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine, and the assessment of the titres of neutralising, anti-RBD, and anti-spike antibodies detected by ELISA Any reported adverse events and the diary book of the participants will be reviewed, and all required information will be recorded.

Visit 7

Phase I and Phase II (118±7)

All participants will undergo a thorough physical examination, and a drug history will be obtained. Blood and urine sampling will be gathered for CBC with a differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine, and the assessment of the titres of neutralising, anti-RBD, and anti-spike antibodies detected by ELISA. Any reported adverse events and the diary book of the participants will be reviewed, and all required information will be recorded.

Visit 8

Phase I and Phase II (Day 208±7)

All participants will undergo a thorough physical examination, and a drug history will be obtained. Blood and urine sampling will be gathered for CBC with a differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine, and the assessment of the titres of neutralising, anti-RBD, and anti-spike antibodies detected by ELISA. Any reported adverse events and the diary book of the participants will be reviewed, and all required information will be recorded.

Visit 9

Phase I and Phase II (388 ±7)

All participants will undergo a thorough physical examination, and a drug history will be obtained. Blood and urine sampling will be gathered for CBC with a differential test, liver function and blood biochemistry test, urine analysis, lymphocyte subset, cytokine, and the assessment of the titres of neutralising, anti-RBD, and anti-spike antibodies detected by ELISA. Any reported adverse events and the diary book of the participants will be reviewed, and all required information will be recorded.

9. Safety assessments

Safety assessments included monitoring and recording of local and systemic solicited (Day 0 to 7 after vaccination) and unsolicited (Day 8 to 28 after vaccination) adverse events (AEs); AEs of special interest (AESI); serious AEs (SAEs); clinical laboratory results including haematology and serum chemistry; vital sign measurements; and physical examination findings. Relatedness/causality and severity grading is also based on the World Health Organization (WHO) causality assessment. Vaccination pauses rules based on reactogenicity, safety laboratory results, and SAEs related to study participation are in place to monitor subject safety during the study.

Adverse event or adverse experience

Safety is assessed based on reports of adverse events, laboratory test results, and vital signs measurements. The AEs examined in this study are as follows:

1) Local clinical complications: pain, erythema, swelling, itching
2) Systemic clinical complications: fever, diarrhoea, constipation, dysphagia, anorexia, fatigue, nausea, vomiting, myalgia, arthralgia, headache, cough, shortness of breath, itching at the injection site, acute allergic reaction
3) Other complications include any increase in liver enzymes, changes in measured biochemical parameters, lymphopenia, leukocytosis or

leukopenia, neutropenia, platelet depletion, eosinophilia, hyperglycemia, sugar, protein, or RBC in urine

Solicited AEs	All AEs occurred during days 0 to 7 after administration
Unsolicited AEs	All AEs occurred during days 8 to 28 after administration

Complications associated with Enhanced Respiratory Disease (ERD)

Enhanced respiratory disease (ERD) or Vaccine-associated enhanced respiratory disease (VAERD) refers to an adverse event where an exacerbated course of respiratory disease occurs with a higher incidence in the vaccinated population than the control group. Considering the significance of ERD in clinical trials regarding viral vaccine interventions, special attention has been paid to it. However, the occurrence of current side effects has not been yet proven among various anti-SARS-COV-2 vaccines. FDA guideline on vaccines against diseases recommends that in cases of suspicion for ERD, animal studies must be closely monitored for evaluating the neutralising antibody production and animal challenge. These measures have been performed in the present study, and preclinical results have been presented. As it is impossible to ensure ERD occurrence in human studies during sufficient time and in large numbers of people, considering phase 3 clinical trials is required. It is worth mentioning that monitoring the following measurements would enormously assist outcome assessors in determining the likelihood of ERD. Two cardinal steps in monitoring ERD are as follows:

1) The amount of neutralising antibody in various measurements: As not increasing the amount, the probability of ERD is more substantial.
2) More T-helper type 2 (Th-2) response than T-helper type 1 (Th-1): As reflected in the corresponding interleukins and eosinophils: Th1 produces interleukins 2 and 12, as well as IFN gamma and TNF alpha. While Th2

produces allergic responses such as an increased eosinophil ratio, it produces interleukins 4, 5, 6, 10, 13, and 25.

ERD will be monitored in the current study by evaluating the values during the study, including short-term (up to 28 days) and long-term (in months 3, 6, and 12). These reviews will be conducted by the Data Security Committee during formal meetings.

Any unwanted events must be recorded. If the effect is severe enough to require medical attention, it should be reported as soon as possible and within a few days. The Data and Safety Monitoring Board (DSMB) should be notified and decide whether blinding will continue and whether a participant will be excluded from the study. In case of severe or fatal events that require hospitalization, reporting should be done promptly and by fax to the person in charge of Shifa Pharmed Industrial Group on the same day.

Definition of an adverse event

An adverse event (AE) is defined as any medical event among participants who participated in the trial regardless of being in the intervention or placebo group and is different from the clinical manifestations of disease progression and does not necessarily have a causal relationship with the study intervention. Clinical manifestations that begin as an adverse event include any signs, symptoms (as any abnormal laboratory diagnosis), or a temporary illness associated with drug use in the study, regardless of whether it is etiologically related.

A clinical condition that is present before the start of the study is considered an adverse event only if it worsens during the study and is not attributable to the normal progression of the disease.

Adverse drug reaction (ADR)

Adverse drug response (ADR) is an adverse drug response to conventional doses used in humans to prevent, diagnose, and treat disease or alter physiological function.

Definition of a serious adverse event

Any consequence that results in death is a risk of death, requires hospitalization, prolongs previous hospitalizations, leads to persistent or significant disability, causes malformation or congenital malformations, and requires surgical intervention or medical intervention to avoid permanent injury is considered as serious adverse outcome (SAE).

Recording of adverse events, adverse drug reactions, and serious adverse events

All AEs and ADRs are recorded in the participating medical records and appropriate CRF sections. They are classified according to their severity and relationship to the study treatment according to the criteria of the researchers and the guideline discussed below.

AE is Classified based on the severity of AE according to the standard guideline in the Common Toxicity Criteria (Common Toxicity Criteria, version 4.03, published on June 14, 2010).

All AEs that are not mentioned in the guideline will be classified as mild, moderate, or severe:

1) Mild: Any reaction, sign, or symptom that a person may find but does not interfere with a person's everyday activities and routine
2) Moderate: Any reaction, sign, or symptom that is annoying enough to interfere with a person's normal daily activities. And may require medical intervention
3) Severe: Any reaction, symptom, or symptom that causes a great deal of discomfort that significantly interferes with a person's daily activities and

poses a specific disability or health risk. These cases usually require medical intervention

To make the connection between AE or ADR and treatment, the following definition will be considered in the study:

Certain: A clinical outcome involving changes in laboratory tests with reasonable temporary manifestations associated with drug administration that cannot be explained by current illness, medication, or other substances. The response to drug suppression should be clinically reasonable and plausible.

Probable: A clinical outcome involving a change in laboratory tests that presents with a temporary logical sequence that is related to the prescription of the drug and is unlikely to be attributable to the previous disease or other substances and drugs, and those Clinical rational responses occur when the drug is stopped (dechallenge). Rechallenge information is not required for this definition.

Possible: A clinical consequence involving a change in laboratory tests that manifests itself in a temporary logical sequence related to the drug administration and also explained by another concomitant disease or other substances and drugs. Discontinuation information may be missing or unclear.

Improbable: A clinical outcome involving a change in laboratory tests that presents with a temporary logical sequence related to the drug administration and could be explained more logically with another concomitant disease or other substances and drugs.

Conditional / Unclassified: A clinical outcome involving changes in laboratory tests is reported as an AE, which is necessary to obtain more data for proper evaluation, or more data is under review.

Unassessable / Unclassifiable: A report that presents an unwanted reaction but cannot be judged because the information is insufficient or inconsistent and cannot be confirmed or completed with the relevant data.

Diary book

Participants will be required to complete a daily reactogenicity diary book through provided notebooks. All participants in Phase 1 and Phase 2 will be asked to monitor and record local reactions, systemic events, and antipyretic medication usage for seven days following administration of the study intervention. The reactogenicity diary book allows recording of these assessments only within a fixed time window, thus providing an accurate representation of the participant's experience at that time. Data on local reactions and systemic events reported in the reactogenicity diary book will be transferred to a third party, where they will be available for review by outcome assessors and clinicians at all times.

Outcome assessors (or designees) will be required to review the reactogenicity diary book data at frequent intervals as part of the ongoing safety review.

The outcome assessor or designee must obtain stop dates from the participant for any ongoing local reactions, systemic events, or use of antipyretic medication on the last day that the reactogenicity diary book was completed. The stop dates should be documented in the source documents and the information entered in the CRF.

Call centre

All Phase I and Phase II participants will be capable of contacting the 24/7 study call centre should they have any concerns or need medical attention.

Laboratory assessments

The following safety laboratory tests will be performed at pre-determined times. Additional laboratory results may be reported on these samples due to the analysis

method or the type of analyzer used by the clinical laboratory or as derived from calculated values. These additional tests would not require a further collection of blood. Unscheduled clinical laboratory measurements may be obtained during the study to assess any perceived safety issues.

Haematology	Biochemistry	Other
Haemoglobin Hematocrit RBC count MCV MCH MCHC Platelet count WBC count Total neutrophils (Abs) Eosinophils (Abs) Monocytes (Abs) Basophils (Abs) Lymphocytes (Abs)	BUN and creatinine AST, ALT Total bilirubin Alkaline phosphatase	Urine pregnancy test (β-hCG) **At screening only:** Hepatitis B core antibody Hepatitis B surface antigen Hepatitis C antibody Human immunodeficiency virus

Grading

Grading is based on the FDA Guidelines for Toxicity Rating in Healthy Individuals Participating in Vaccine Studies.

Adverse Event	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Local Reaction to Injectable Product				
Pain	Does not interfere with the activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with the activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization

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Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness[1]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling[2]	2.5 – 5 cm and does not interfere with the activity	5.1 – 10 cm or interferes with the activity	> 10 cm or prevents daily activity	Necrosis
Vital Signs [3]				
Fever (°C) [4] (°F) [4]	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute[5]	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation
Systemic (General) reactions to Injectable Product				
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhoea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	Six or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some	Significant; any use of narcotic pain reliever or	ER visit or hospitalization

		interference with activity	prevents daily activity	
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Systemic Illness				
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

[1] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
[2] Induration/Swelling should be evaluated and graded using the functional scale and the actual measurement.
[3] Subject should be at rest for all vital sign measurements.
[4] Oral temperature; no recent hot or cold beverages or smoking.
[5] When resting, heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Grading scales for laboratory findings are as follows:

Abnormality	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)[2]**
Serum[1]				
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia	100 – 110	111 – 125	>125	Insulin requirements or

Fasting – mg/dL Random – mg/dL	110 – 125	126 – 200	>200	hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN[3]	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN
Hematology [4]				
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female)	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0

change from baseline value - gm/dL				
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN[3]	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)
Urine [5]				
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 - 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

[1] The laboratory values provided in the tables serve as guidelines and are dependent upon normal institutional parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
[2] The clinical signs or symptoms associated with laboratory abnormalities might result in the characterization of the laboratory abnormalities as Potentially Life-Threatening (Grade 4). For example. A low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.
[3] ULN" is the upper limit of the normal range.
[4] The laboratory values provided in the tables serve as guidelines and are dependent upon normal institutional parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
[5] The laboratory values provided in the tables serve as guidelines and are dependent upon normal institutional parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Action taken

All undesirable and unexpected AEs after the vaccine candidate or placebo administration will be accurately recorded in the volunteer medical file and the relevant CRF section. The side effects will be reported according to the Food and Drug Administration guidelines on reporting safety during clinical studies.

Follow-up of adverse events, adverse drug reactions, and serious adverse events

The outcome assessor is required to follow up with all participants in case of any complication until the symptoms disappear entirely or the patient's condition returns to normal.

Reporting of all serious adverse events

The principal outcome assessor is required to immediately inform the representative of the pharmaceutical company in the cases of any severe adverse events that result in the death or risk of death of the participant within 24 hours. In addition, the National Ethics Committee/Supervisor will be appointed by the Ethics Committee, and the General Directorate of Drugs/Supervisor announces the Food and Drug Administration.

In the cases of no risk of death of the participant with SAE, the outcome assessor is required to inform the representative of the pharmaceutical company, the National Ethics Committee / Ethics Committee, and General Directorate of Drugs /Food and Drug Administration Supervisor within a maximum of 48 hours according to the study policies (Which is seven calendar days according to the national instructions).

If an adverse event at SUSAR results in the participant's death or risk of death, Shifa-Pharmed Company shall be informed in the shortest possible time (maximum 48 hours according to the May study policy, of course, seven days

specified in the national guidelines). The event announces the matter using the relevant CIOMS form for the National Ethics Committee / Supervisor of the Ethics Committee and the General Directorate of Drugs / Supervisor of the Food and Drug Administration. The Company's supplementary report on the relationship between the complication and the study vaccine will be submitted within a maximum of 5 calendar days according to the study policy (15 days specified in the national guidelines) after the event.

Occurrence of any Suspected Unexpected Serious Adverse Reaction (SUSARs) without any risk of death, which is susceptible to exacerbation or requires medical intervention to prevent life-threatening events Pharmed Company must be notified in the shortest time (the basis of the study policies, which is 15 days according to the national guidelines). The company's supplementary report on the association of the complication with the study vaccine will be submitted within 48 hours (according to the study policy, which is 15 calendar days according to national guidelines) after the event.

Finally, all severe adverse events and follow-up results will be reported to the General Directorate of Drugs within five days after the company is aware of the SAE.

Predictable events, of which the severity and frequency of complication occurrence led to the withdrawal of the participants, or the number of the participants with complication is higher than expected, Pharmacy Company reports the matter to the General Directorate of Drugs within five calendar days. The company notifies the Food and Drug Administration team or DSMB committee to evaluate the cases regarding the possibility of increased risk within a maximum of 5 calendar days.

Data safety monitoring board (DSMB)

Data safety monitoring board (DSMB) as an independent committee during pre-determined sessions, which aimed to ensure the safety of the research product (Safety review) and the progress process, periodically will collect and review the safety and effectiveness of the product. The committee will decide whether to increase the high dose for participants after the arrival of the first 14 participants receiving a low dose or placebo to review the overall safety.

Stage I- Phase I:

Meeting	Time	The main objective
1	Start of study (before participants arrive)	Coordination and decisions to start the study
2	After the arrival of the initial three participants (Get a low-dose vaccine or placebo)	Check for immediate and safe side effects at the beginning of the study
3	4 days after the arrival of the initial 7 participants (Get a low-dose vaccine or placebo)	Evaluation of immediate and safety effects and laboratory results at the beginning of the study
4	8 days after the arrival of the initial 14 participants (Get a low-dose vaccine or placebo)	Safety assessment and laboratory results at the beginning of the study Decide whether to start the intervention with high dose (5µg) vaccine
5	7 days after the arrival of 21 participants (Get a low-dose vaccine or placebo)	Evaluation of the safety and immediate side effects and initial laboratory results in high dose (5µg) Evaluation of safety in low-dose vaccine booster dose (3µg)
6	14 days after arrival, 35 participants (Get a low-dose vaccine or placebo)	Evaluation of safety in booster dose of low and high dose vaccine (3 and 5µg)
7	28 days after the arrival of 56 participants (Total participants)	Evaluation of safety and immunogenicity in all participants and final decision

Stage II- Phase I:

Meeting	Time	The main objective
1	After the arrival of the initial 8 participants	Check for immediate and safe side effects

	(6 for the selected dosage of vaccine and 2 for placebo)	at the beginning of the study
2	After the arrival of 24 participants	Evaluation of safety and immunogenicity in all participants and final decision

Phase II:

Meeting	Time	The main objective
1	14 days after both injections of 35% of participants in Phase II	Review of the study process and announcement of the considerations related to continuing the study, review of the safety of the volunteers
2	14 days after both injections of all participants in Phase II	Review of the study process and announcement of the considerations related to continuing the study, review of the safety of the volunteers
3	After completing the 90-day follow-up period, 30% of the participants entered the study in phase three	Review of the study process and announcement of the considerations related to continuing the study, review of the safety and effectiveness of the volunteers
4	After completing the 90-day follow-up period, 50% of the participants entered the study in phase three	Review of the study process and announcement of the considerations related to continuing the study, review of the safety and effectiveness of the volunteers
5	After completing the 90-day follow-up period, 70% of the participants entered the study in phase three	Review of the study process and announcement of the considerations related to continuing the study, review of the safety and effectiveness of the volunteers
6	After completing the 90-day follow-up period, 100% of the participants entered the study in phase three	Review of the study process and announcement of the considerations related to continuing the study, review of the safety and effectiveness of the volunteers

Considering the AEs occurred among participants in the study (i.e. the occurrence of severe complications), DSMB might decide to increase the frequency of the

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meetings. The meetings of this committee will be held online or in-person with the presence of the sponsor's representative, the principal investigator, the independent members of the committee, and the regulatory representatives.

The committee at any stage will be capable of deciding to continue, make any proposed changes, stop or suspend the study.

10.Immunogenicity assessments

Blood samples for immunogenicity assessments will be collected before each vaccination and at selected time points following the first and second vaccination. Immunogenicity outcomes will be categorised based on humoral responses to the vaccine. The humoral response will be assessed through geometric mean titers (GMT), geometric mean ratios (GMR) of antibodies against SARS-CoV-2, and seroconversion rate. Neutralising, anti-receptor binding domain (RBD), and anti-spike glycoprotein antibodies will be measured using ELISA kits: SARS-CoV-2 Neutralising Ab IgG-96 [14], SARS-CoV-2 RBD IgG-96 [15], and SARS-CoV-2 spike IgG-96 [16], Pishtaz Teb, Tehran, Iran. Moreover, antibodies against the S1 domain of the spike glycoprotein of SARS-CoV-2 will be assessed via EI 2606-9601 G kit, Euroimmun [17]. Seroconversion is defined as an increase in antibodies ≥four times their baseline level.

Conventional Virus Neutralisation Test (cVNT) assay will be employed to assess the vaccine effectiveness in inducing functional antibodies against SARS-CoV-2. To inactivate the complement, plasma samples will be heated at 56°C for 30 minutes. Afterwards, plasma samples will be serially diluted in two-fold dilutions. SARS-CoV-2 suspensions at 100 (Tissue Culture Infectious Dose 50 assay) TCID50 will be incubated with diluted plasma at 37°C and 5% CO2 for an hour. Monolayer Vero E6 cells with 80% confluency will be overlaid with plasma-virus suspensions. Each neutralisation test will be performed in triplicates. Then, virus-specific cytopathic effects (CPE) will be visualised 72

hours later and observed via light microscopy. Neutralising antibody titers will be presented as values of the highest dilution inhibiting CPE formation [18,19].

Safety Monitoring:

All participants were monitored closely for about 30 minutes after inoculation and were accommodated in the study site for seven days.

11.Discontinuation of study intervention/participation

Early discontinuation of the trial

Following the occurrence of any of the following conditions, the clinical trial should be completed before completion:

1) Any realisation of the vaccine candidate's potential safety risks or the quality issues in study design and setting that requires a thorough review and revision by the sponsor
2) Any realisation of the vaccine candidate's potential safety risks or the quality issues in study design and setting that requires a thorough review and revision by the DSMB committee
3) Any requests from the ethics committee regarding the termination of the study due to morality issues
4) A request of relevant regulatory authorities for termination

Study suspension policies

The study must be suspended following each of the conditions described below. In the meantime, the outcome assessor, sponsor, DSMB, and the ethics committee will have a joint meeting in advance to decide whether to terminate the clinical study. It is worth mentioning that the DSMB meeting is required in each of the following cases.

1) Any grade 4 adverse reaction in any group (Grading is based on the FDA Guidelines for Toxicity Rating in Healthy Individuals Participating in Vaccine Studies)
2) Any suspected unexpected serious adverse reaction (SUSAR) related to vaccination occurring in any group
3) The number of participants with Grade 3 adverse reactions among participants of each subgroup after each dose is more than 15% of the total participants by the time of each DSMB session (graded according to the FDA Guide to Toxicity Grading In healthy people who participate in vaccine studies)
4) DSMB evaluates the clinical trial and concludes that there is a high potential risk to safety

Lost to follow-up subjects

Eligible volunteers recruited in the trials might be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing them from receiving the second dose of intervention are as follows:

6) Positive pregnancy test (Beta-HCG) before the second administration
7) Presenting with temperature over 39ºC over three days or any severe allergic reaction after the first inoculation;
8) Reporting any severe adverse events after first administration;
9) Receiving immunoglobulin or steroidal hormones (oral or intravenous) up to two weeks before the second administration;

Withdrawal of the participants from the study will be potentially due to:

10) Refusing to continue the study;
11) Any suspicion of immunodeficiency disorders with physical examination (the laboratory tests are not obligatory);

12) Getting pregnant before the administration of the second dose of vaccine candidate or placebo;
13) Occurrence of severe hypersensitivity reactions (severe anaphylactic or anaphylactoid, bronchospasm, and severe urticaria) to intervention during 30 minutes after administration
14) Administration of other vaccines or forbidden medicines based on trial protocol;
15) Occurrence of a serious adverse event which may convince the PI to withdraw the participant from the study;
16) Any occurrence of an acute or chronic situation which may convince the PI to withdraw the participant from the study;
17) Any deviation from the scheduled visit times based on protocol; and
18) Participation in any other clinical trials.

All participants who met the discontinuation criteria must be considered for the immediate assessment and early termination as soon as possible after the discontinuation. The outcome assessor has to record all justifications of permanent discontinuation or dropout. In case of study discontinuation or drop-out from the study due to the reason mentioned above, the study result will be collected, reviewed, and included in the final assessment.

12.Statistical consideration

Study profile

The final statistical report will include all participants who have signed the informed consent form. The flowchart recommended by CONSORT will be used to show the presence of participants from the moment of admission to clinical trial (screening and review of criteria) to the end of the study (evaluation of study outcomes). The number (percentage) of participants in each treatment group will

be reported for the population per protocol (PP), and the reasons for withdrawal or severe deviations from the protocol will be stated.

Statistical and Analytical Plans

General principles of analysis

All data collected from the Phase I and Phase II studies will be analyzed I based on three analysis populations: Safety population, Intention-To-Treat (ITT) population, and Population per-protocol (PP).

1) **Safety population:** Participants enrolled in the study will receive at least one dose of the study drug.
2) **ITT population**: Participants who have at least one antibody titer measurement following the administration.
3) **PP population**: Participants who will not have any significant deviations from study protocol basics.

Demography and clinical medical history

To identify any statistical difference between the two groups in terms of demography and health status, descriptive statistics (including mean, median, standard deviation, minimum, maximum, etc.) will be calculated, and for continuous variables,, frequencies and percentages will be considered. Concomitant medications will be summarized by treatment group and preferred drug name as coded using the World Health Organization drug dictionary.

Safety analyses

The safety assessments will be performed on the safety population regarding AEs, vital signs, and the results of laboratory tests.

Adverse events

Numbers and percentages (Clopper-Pearson method) of participants with solicited local and systemic AEs (based on the FDA toxicity grading scale) through 7 days after each administration will be summarized. After each

administration, the duration of solicited local and systemic AEs was presented individually.

Unsolicited AEs will be coded by preferred term (PT) and system organ class (SOC) using the latest version of MedDRA and summarized by the treatment group. AEs related to the study vaccine will be defined for those considered as "certain", "probable", or "possible" based on the WHO Causality Assessment.

Adverse events through 28 days after first administration; and SAE, or AESI through 360 days after final vaccination will be listed separately and summarized by treatment group.

Actual values, changes from baseline (where indicated), and toxicity grading for clinical safety laboratory test results and vital sign measurements will be summarized by the treatment group at each time point using descriptive statistics.

Vital signs

Vital signs will be measured on screening day, days 0, 7, 14, 21, and 28 and summarized using descriptive statistics (mean, standard deviation, median). A paired t-test will be used to identify any change in vital signs from pre-vaccination levels within each group.

Results of laboratory test

Changes in laboratory test results before and after vaccination will be analyzed using the paired t-test or McNemar test. If any clinically significant change exists, t-test or GEE analysis will be used to determine if there is a statistically significant difference between treatment groups.

Immunogenicity analysis

Immunogenicity tests will be performed on the data of participants who have received at least one dose of the vaccine/placebo and have the results of measuring blood biomarkers before and after the administration. The missing values will be entered based on the latest observations.

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The geometric mean of the antibody titers and their 95% confidence interval will be calculated after Log Transformation. For stratified data, $\chi 2$ test or Fisher's exact test will be used, and for antibody titer analysis between vaccine and placebo groups, t test will be used.

Determination of Sample Size

The sample size for this study will be based on clinical and practical considerations and not on a formal statistical power calculation. The sample size is considered sufficient to evaluate the objectives of the study. The ratio of vaccination to placebo will be 3:3:1, containing 3µg or 5µg whole virus particle or placebo, in Stage I-Phase I; 3:1, containing 5µg whole virus particle or placebo, in Stage II-Phase I; and 4:1, containing 5µg whole virus particle or placebo, in Phase II.

13.Supporting documentation

Quality control and quality assurance

The principal investigator and sub-investigators will be adequately trained and instructed about the conduct of the study, the study protocol, and GCP guideline by the CRO. In addition, the data recording and handling should be managed by the CRO for more efficient progress.

Only the outcome assessor responsible for completing the CRFs is permitted to correct a case report form. When a correction to an entry needs to be made, a single line is crossed through the data to keep the original entry visible. An initial or signature of a person making a correction and the correction date will be entered beside the correction.

During the study period, a study outcome assessor will be designated by the sponsor to periodically perform monitoring visits to verify if the study is being

conducted according to the study protocol and applicable regulations related to clinical study conduct. In addition, the CRO will perform predefined monitoring visits, which should be shared with the regulatory bodies. An outcome assessor will review source documents and case report forms. If any discrepancy or missing data is found, the outcome assessor will request to make necessary corrections or to provide appropriate documents.

To prevent any error during data entry, the study data in a CRF is double entered (double-entry method). The two databases will be compared (unmatched check) each other for the discrepancy. The database is reviewed to check the logical consistency and compare against the data contained in CRFs (manual check) to ensure the data's accuracy and integrity.

In addition, a data manager will review the case report forms to verify the data consistency or the presence of any missing or unclear entries. A data manager also will generate DCF (Data Clarification Form), if necessary, and request an outcome assessor to review. Data correction and re-entry are carried out under the supervision of an outcome assessor and the data manager. Only designated persons are allowed access to the database for the data entry and correcting. All entries and a record of corrections made are retained.

Monitoring

Data quality control objectives include the following:

- Ensuring the completion of signed written consent forms
- Identify issues and problems (especially systematic cases) as soon as possible to provide appropriate operational and corrective plans
- Ensure data validity

To achieve these goals, quality control is done through the following activities:

- A meeting is held before the start of the study with the executive team and researchers at the leading site of the patient (Eram Hotel). The study

protocol will be reviewed, the implementation process will be re-explained, the volunteer entry site will be visited, and the workflow for the first volunteer entry will be run as role-playing.

- Quality control will be performed daily, and its daily report will be recorded and maintained

Quality control will be done at two levels:

- Quality of completing data collection forms (CRF) and entering information into the eCRF system: When receiving CRF, the local study team of the same site reviews the completed forms to ensure their completeness and quality assurance. The site study team (the outcome assessor or their representative) is responsible for providing an action plan to improve the site's quality. In the event of systematic problems, the local study team will notify the company's study team.
- Central database review: In addition to the standard measures available for data management, the following parameters will be evaluated daily by the central study team as part of quality control at the study, site, and country-level:

- Checking the box related to the informed consent form
- The amount of erroneous and missing data for the critical study variables
- Completing the participant questionnaire form

The monitor should complete a quality control visit report that includes a quality control questionnaire during a quality control visit.

The principal outcome assessor should make the necessary arrangements for quality control of the following:

- Signed informed consent form
- Delivery, storage, and transportation of research products
- All clinical examinations
- Laboratory tests and other paraclinical examinations

- Participant care

Ethical considerations

Independent ethics committee (IEC) or institutional review board (IRB)

Before initiation, this clinical study and all study-related documents are approved by the Iran Food and Drug Administration (IFDA), including the study protocol, subject information sheets, and subject informed consent form. In addition, all documents related to the study, including the study protocol, are also approved by the National Ethics Committee (NEC) of Iran (IR.NREC.1399.003 and IR.NREC.1399.007 for Phase I, and IR.NREC.1399.008 for Phase II) before initiation of the study.

Ethical conduct of the study

The overall study procedures related to conducting the study, record retention, data collection, and application process for the approval of IFDA are carried out in compliance with Good Clinical Practice (GCP) and the standard operation procedure (SOP) of the Shifa Pharmed Industrial Group. They are conducted in accordance with the principles that have their origin in the Declaration of Helsinki to ensure the right and safety of study subjects.

Subject information and consent

The principal investigator and sub-investigators clarify the study's characteristics, scope, and anticipated outcomes in layman's terms to the study participants and obtain written consent to participate in the study. There are two types of informed consent forms; one before screening and another before allocation. The consent forms will be signed in person by the volunteer and by the principal investigator (or an investigator delegated with obtaining the consent form). After the written informed consent is given, copies of the signed consent form and subject information sheets will be provided to the volunteer. The sponsor prepared and provided the informed consent form to the principal investigator (sub-investigators). The names of all volunteers will remain confidential, and they will

be identified only by their initial screening number and randomization number during the data recording or assessment. All volunteers will be informed that all study data would be handled strictly confidential. The signed informed consent forms will be retained in the study centre after completing the study.

Responsibilities of principal (site) investigator

All determined responsibilities of the Principal Investigator in this study are as follows:

1) Delicately Carrying out the study in accordance with the protocol approved by the Iran Food and Drug Administration and other regulatory authorities;
2) Forming and organizing a research team;
3) Organizing the training sessions for executive team members and new hires when needed;
4) Monitoring the availability of a suitable place and space for the reception of participants;
5) Supervision of the availability of an appropriate warehouse for research products used in the clinical trial
6) Effective collaboration with and providing access to all study documents for study monitors and designated observers during the study
7) Providing individual case report of the occurrence of severe adverse events, including deaths, to the General Directorate of Iran Food and Drug Administration and the National Ethics Committee in accordance with the study protocol and national laws
8) All documents related to the trial must be submitted to Shifa Pharmed Industrial Group at the end of the study. The original executor might keep a copy (according to national law).

No part of the trial should be published without the prior consent of the sponsoring company.

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Data management

The CRO is responsible for maintaining all forms and documentation related to the participants over time. Original copies of CRFs must be submitted to the Data Management Center at the end of the study at the request of the data management team and sponsor approval. The original copies should be delivered to the data management department at the end of the study. A copy of the documentation could be kept in the study centre. The process of sending and receiving all documents will comply with all security and safety principles. It is worth mentioning that supervision of the correct execution of all processes is the responsibility of the PI.

Confidentiality

Any study-related information will be kept confidential at the study site. All participants' data will be stored on locked shelves in a place with limited access. All laboratory samples, reports, data collection forms, and executive processes of the participants are marked with confidential codes. All forms containing the participant's name and other identifying information, such as informed consent forms, will be kept separately from restricted research forms with specific codes in places with limited access. Databases will be protected with secure passwords. Forms, lists and logbooks, appointments, and other lists that link the participant ID number to other information are stored in a locked file in a restricted location. All laboratory results and examinations of the participants are kept entirely confidential, and all research staff is required to sign and observe the principles of confidentiality of all study participants.

Publication policy

No other publication will be allowed before the initial publication of the results. The sponsor coordinates any presentation or publication following the preliminary results and is based on the initial report results.

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15.List of abbreviations and definitions of the term

ADR	Adverse Drug Reaction
AE	Adverse Event
ANOVA	Analysis of variance
Anti-RBD	Anti-receptor binding domain
BMI	Body Mass Index
BSL-3	Biosafety level 3
CBC	Complete Blood Count
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Corona Virus Disease 2019
CRF	Case report form
CRO	Contract Research Organization
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked ImmunoSorbent Assay
FAS	Full analysis set population
GCP	Good Clinical Practice
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titres
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICD	International Classification of Diseases
IEC	Independent Ethics Committee
IRB	Institutional review board
IFDA	Iran Food and Drug Administration
IFN-gamma	Interferon-Gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interlukin
IRB	Institutional Review Board
IRCT	Iranian Registry of Clinical Trials
ITT	Intention to treat
LFT	Liver function test
MedDRA	Medical Dictionary for Regulatory Activities
NEC	National Ethics Committee
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs

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PI	Principal investigator
PPS	Per protocol set
RT-PCR	Reverse Transcriptase- Polymerase Chain Reaction
SAE	Serious Adverse Event
SARS-COV-2	Severe Acute Respiratory Syndrome- Corona virus- 2
SAH	Single human dose
SPSS	Statistical Product and Service Solutions
SUSAR	Suspected Unexpected Serious Adverse Reaction
Th-1	T-helper type 1
Th-2	T-helper type 2
TNF-alpha	Tumor Necrosis Factor-Alpha
WHO	World Health Organization
$\chi 2$	Chi-Square test
SOP	Standard Operating Procedure
AE	Adverse Event
AESI	Adverse Event Of Special Interest
SAE	Serious Adverse Event
DCF	Data Clarification Form
ITT	Intent-to-treat
PPS	Per Protocol Set

Supplemental appendix 2

Supplement to: Safety and immunogenicity of an inactivated virus particle vaccine for SARS-CoV-2, BIV1-CovIran: findings from double-blind, randomised, placebo-controlled, phase I and II clinical trials among healthy adults

Mohraz M, *et al. BMJ Open* 2022; 12:e056872. doi: 10.1136/bmjopen-2021-056872

Table S1. Solicited and unsolicited adverse events among participants aged 18-50 years in Phase I

Adverse events		Solicited AEs						Unsolicited AEs					
		First administration n(%)			Second administration n(%)			First administration n(%)			Second administration n(%)		
		Placebo	3 µg	5 µg	Placebo	3 µg	5 µg	Placebo	3 µg	5 µg	Placebo	3 µg	5 µg
Injection site involvement	Pain in the injection site	2(25.0)	6(25.0)	8(33.3)	4(50.0)	7(30.4)	8(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
	Induration/Swelling in the injection site	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	2(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Erythema/Redness in the injection site	1(12.5)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	2(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
General reactions	Fatigue	1(12.5)	1(4.2)	0(0.0)	0(0.0)	2(8.7)	5(20.8)	1(12.5)	2(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Myalgia	0(0.0)	6(25.0)	3(12.5)	1(12.5)	5(21.7)	7(29.2)	0(0.0)	3(12.5)	0(0.0)	0(0.0)	3(13)	3(12.5)
	Fever	2(25)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Chills	0(0.0)	0(0.0)	2(8.3)	0(0.0)	0(0.0)	2(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Flushing	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
Upper and lower respiratory system disorders	Dyspnea	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Tachypnea	1(12.5)	4(16.7)	0(0.0)	1(12.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Coughing	0(0.0)	0(0.0)	1(4.2)	1(12.5)	1(4.3)	2(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Sore Throat	1(12.5)	0(0.0)	0(0.0)	1(12.5)	0(0.0)	1(4.2)	0(0.0)	1(4.2)	0(0.0)	1(12.5)	1(4.3)	0(0.0)
	Rhinitis	1(12.5)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	2(8.3)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	1(4.3)	0(0.0)
	Chest pain	1(12.5)	1(4.2)	1(4.2)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	2(8.3)	0(0.0)	0(0.0)	1(4.2)
Gastrointestinal, stomach and urinary disorders	Nausea/Vomiting	0(0.0)	0(0.0)	2(8.3)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	1(12.5)	0(0.0)	1(4.2)
	Abdominal Pain	0(0.0)	1(4.2)	2(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
	Diarrhea	0(0.0)	0(0.0)	1(4.2)	0(0.0)	2(8.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
	Renal pain	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Skin and subcutaneous tissue disorders	Pruritus	0(0.0)	2(8.3)	2(8.3)	1(12.5)	2(8.7)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
	Erythema	0(0.0)	1(4.2)	0(0.0)	1(12.5)	2(8.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Hair loss	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(12.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Cardiovascular system disorders	Tachycardia	1(12.5)	1(4.2)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Hypotension (systolic)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Nervous system disorders	Headache	3(37.5)	8(33.3)	6(25.0)	2(25.0)	3(13)	7(29.2)	1(12.5)	1(4.2)	0(0.0)	0(0.0)	1(4.3)	2(8.3)
	Dizziness	0(0.0)	2(8.3)	0(0.0)	0(0.0)	2(8.7)	2(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
	Paresthesia	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	2(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Dysphonia	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Eye involvement	Periorbital Oedema	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Abnormal vision	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Abnormal sensation in eye	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Infections and infestations	Herpes simplex	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Table S2. Solicited and unsolicited adverse events among participants aged 51-75 years in Phase I

Adverse events		Solicited AEs				Unsolicited AEs			
		First administration n(%)		Second administration n(%)		First administration n(%)		Second administration n(%)	
		Placebo	5 μg	Placebo	5 μg	Placebo	5 μg	Placebo	5 μg
Injection site involvement	Pain in the injection site	1(12.5)	8(34.8)	2(25)	6(26.1)	0(0.0)	0(0.0)	0(0.0)	1(4.4)
General reactions	Fever	0(0.0)	0(0.0)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Weakness	0(0.0)	1(4.4)	0(0.0)	3(13.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Fatigue	0(0.0)	0(0.0)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Myalgia	0(0.0)	0(0.0)	1(12.5)	2(8.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Flushing	1(12.5)	2(8.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Increased sweating	0(0.0)	0(0.0)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Upper and lower respiratory system disorders	Rhinitis	0(0.0)	0(0.0)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Coughing	0(0.0)	0(0.0)	0(0.0)	5(21.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Sore Throat	0(0.0)	1(4.4)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	1(4.4)
	Tachypnea	0(0.0)	0(0.0)	0(0.0)	3(13.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Gastrointestinal, stomach and urinary disorders	Diarrhea	0(0.0)	2(8.7)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	2(8.7)
	Nausea	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Abdominal Pain	0(0.0)	0(0.0)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Constipation	0(0.0)	1(4.4)	1(12.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Cardiovascular system disorders	Tachycardia	0(0.0)	1(4.4)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Bradycardia	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.4)	0(0.0)	0(0.0)
	Hypertension (Systolic)	0(0.0)	0(0.0)	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Nervous system disorders	Vertigo	0(0.0)	1(4.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Headache	1(12.5)	2(8.7)	1(12.5)	3(13.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Table S3. Laboratory assessment of the participants aged 18-50 years in Phase I

Test (Unit)	Day	3µg group			5µg group			Placebo group		
		Mean	Range of values	Abnormal values	Mean	Range of values	Abnormal values	Mean	Range of values	Abnormal values
Hematocrit (g/dL)	0	43.7	35.3-51.6	0	43.4	39.9-53.1	0	44.3	42.2-49.4	0
	7	43.4	34.1-50.4	0	43.0	40.4-52.2	0	43.0	42.1-49.5	0
	14	43.1	34.1-50.3	0	43.0	40.5-51.5	0	44.6	39.8-86.0	1
	21	43.3	35.0-49.8	0	42.4	40.0-51.4	0	44.4	39.8-46.8	0
	28	43.1	34.9-50.6	0	43.1	39.4-50.3	0	43.9	40.7-46.6	0
Hemoglobin (g/dL)	0	16.2	11.6-17.4	1	16.0	12.7-17.7	0	14.2	13.3-16.1	0
	7	16.3	11.1-16.9	2	15.9	13.2-16.9	0	13.7	13.3-16.5	0
	14	16.1	11.2-16.9	1	16.0	13.1-17.4	0	14.3	13.1-41.2	1
	21	15.9	11.4-16.9	1	15.8	12.9-16.8	0	14.0	12.9-16.0	0
	28	15.8	11.3-16.9	1	15.8	12.8-16.8	0	13.4	13.0-15.9	0
White Blood Cells (/µliter)	0	6791.7	3660.0-10030.0	2	6400.8	4280.0-9120.0	0	7062.9	3810.0-7450.0	1
	7	6750.8	4000.0-9070.0	0	6746.3	4590.0-9210.0	0	7357.1	3810.0-8800.0	1
	14	6971.7	3200.0-9930.0	1	6995.7	4970.0-9970.0	0	6966.3	5380.0-9080.0	0
	21	7017.4	3880.0-10630.0	1	6771.3	4940.0-9170.0	0	6557.5	5380.0-7750.0	0
	28	6918.2	3560.0-8760.0	2	6397.9	4290.0-9290.0	0	7141.4	3880.0-9270.0	1
Neutrophils (%)	0	58.8	42.9-74.5	0	55.9	41.0-69.7	0	53.0	46.6-60.7	0
	7	57.1	44.0-69.0	0	55.9	40.0-70.1	0	54.5	37.9-60.4	0
	14	55.9	44.6-68.1	0	54.8	41.8-67.0	0	50.1	41.1-62.5	0
	21	58.0	40.0-70.6	0	55.1	44.1-68.7	0	51.3	33.6-66.5	0
	28	58.2	41.0-73.0	0	57.9	37.9-72.0	0	49.0	40.7-61.4	0
Lymphocytes (%)	0	32.5	19.6-38.8	0	33.7	17.3-51.0	0	39.0	30.3-44.0	0
	7	32.7	25.1-46.1	0	34.6	21.8-51.0	0	36.7	30.1-51.7	0
	14	34.1	22.1-43.2	0	36.5	26.7-54.6	0	40.5	29.4-47.6	0
	21	32.2	21.4-49.0	0	33.7	26.0-43.1	0	39.6	25.9-55.4	0
	28	32.4	19.5-50.0	0	32.0	21.9-39.8	0	40.9	29.6-49.2	0
Monocytes (%)	0	6.2	3.5-7.6	0	7.1	4-12.4	0	6.0	4.0-7.6	0
	7	6.9	4.0-8.9	0	5.8	0.2-9.2	0	6.0	4.2-7.5	0
	14	6.7	2.0-9.4	0	6.5	3.0-8.4	0	6.4	5.5-7.0	0
	21	6.5	2.0-9.2	0	7.1	5.0-8.4	0	6.5	5.0-7.3	0
	28	6.0	0.4-8.7	0	6.5	3.0-7.9	0	7.2	5.0-9.8	0
Eosinophils (%)	0	2.4	0.2-5.4	0	2.5	0.4-8.9	0	2.1	0.8-3.5	0
	7	3.1	0.1-10.4	0	3.2	0.7-16.9	0	2.4	1.1-4.2	0
	14	3.2	0.5-10.6	0	3.4	1.0-9.2	0	2.8	1.1-4.7	0
	21	3.7	0.5-6.3	0	3.9	1.0-20.3	0	2.4	1.3-3.5	0
	28	3.3	0.3-15.0	0	3.9	0.7-5.7	0	2.7	1.2-4.3	0
Basophils (%)	0	0.2	0.1-0.5	0	0.3	0.1-1.0	0	0.1	0.1-0.3	0
	7	0.3	0.1-0.6	0	0.4	0.1-2.0	0	0.2	0.1-0.4	0
	14	0.2	0.1-0.3	0	0.2	0.1-0.3	0	0.2	0.1-0.4	0
	21	0.3	0.1-0.5	0	0.2	0.1-0.5	0	0.2	0.1-0.3	0
	28	1.6	0.1-2.0	0	0.4	0.1-3.0	0	0.3	0.1-0.3	·
Platelet count (/µliter)	0	273838.7	162000.0-406000.0	0	258291.7	193000.0-353000.0	0	273500.0	227000.0-353000.0	0
	7	293000.0	176000.0-374000.0	0	268250.0	185000.0-371000.0	0	268125.0	227000.0-351000.0	0
	14	295087.0	178000.0-422000.0	0	273090.9	187000.0-434000.0	0	283750.0	240000.0-348000.0	0
	21	292583.3	182000.0-405000.0	0	277666.7	180000.0-382000.0	0	288125.0	219000.0-338000.0	0
	28	281739.1	160000.0-398000.0	0	263916.7	178000.0-359000.0	0	270500.0	235000.0-290000.0	·
SGOT (U/µliter)	0	20.5	10.0-45.0	1	21.0	14.0-30.0	0	18.9	14.0-23.0	0

	7	20.5	15.0-57.0	3	21.3	14.0-47.0	1	20.0	14.0-30.0	0
	14	19.9	12.0-30.0	0	20.0	13.0-38.0	1	19.5	12.0-25.0	0
	21	20.6	12.0-32.0	0	21.4	12.0-47.0	1	18.1	13.0-26.0	0
	28	NE*	NE	NE	NE	NE	NE	NE	NE	-
	0	18.7	7.0-77.0	3	20.3	4.0-47.0	2	18.0	12.0-31.0	0
	7	20.4	7.7-68.0	3	24.5	7.0-62.0	2	16.6	11.0-42.0	1
SGPT (U/µliter)	14	20.8	9.0-49.0	2	21.5	7.0-55.0	4	20.3	11.0-27.0	0
	21	18.8	7.0-55.0	1	20.4	5.0-42.0	3	17.1	10.0-30.0	0
	28	NE	NE	NE	NE	NE	NE	NE	NE	-
	0	178.0	106.0-374.0	1	185.0	125.0-310.0	1	179.4	93.0-327.0	1
	7	189.5	118.0-288.0	0	188.7	120.0-402.0	1	161.3	64.0-337.0	1
Alkaline phosphatase (U/µliter)	14	176.7	109.0-326.0	1	171.2	33.0-270.0	0	181.9	118.0-277.0	0
	21	194.3	114.0-302.0	0	176.6	118.0-311.0	0	164.1	103.0-238.0	0
	28	NE	NE	NE	NE	NE	NE	NE	NE	-
	0	0.6	0.1-1.5	0	0..58	0.1-1.3	0	0.5	0.19-0.8	0
	7	0.6	0.2-1.4	0	0.6	0.2-1.4	0	0.5	0.3-0.7	0
Bilirubin (mg/dL)	14	0.6	0.2-1.3	0	0.7	0.1-1.4	0	0.5	0.2-1.4	0
	21	0.6	0.3-1.4	0	0.6	0.2-14	0	0.6	0.3-1.0	0
	28	NE	NE	NE	NE	NE	NE	NE	NE	NE
	0	24.1	15.0-34.0	0	25.4	11.0-35.0	0	23.1	19.0-27.0	0
	7	25.8	19.0-33.0	0	29.5	19.0-44.0	0	26.6	21.0-37.0	0
Urea (mg/dL)	14	24.1	11.0-33.0	0	27.0	16.0-48.0	0	23.5	14.0-30.0	0
	21	24.1	13.0-31.0	0	24.5	13.0-31.0	0	22.6	14.0-32.0	0
	28	24.1	15.0-30.0	0	23.4	11.0-35.0	0	23.1	19.0-27.0	·
	0	0.9	0.8-1.25	0	1.0	0.8-1.2	0	1.0	0.86-1.1	0
	7	0.9	0.8-1.2	0	0.9	0.7-1.2	0	1.0	0.8-1.05	0
Creatinine (mg/dl)	14	1.0	0.8-1.2	0	1.0	0.7-1.2	0	0.9	0.8-1.4	0
	21	0.9	0.8-1.2	0	1.0	0.8-1.2	0	1.0	0.8-1.1	0
	28	NE	NE	NE	NE	NE	NE	NE	NE	NE
	0	140.0	137.0-144.0	0	140.3	137.0-144.0	0	139.0	137.0-141.0	0
	7	139.5	136.0-142.0	0	139.3	136.0-144.0	0	139.5	138.0-141.0	0
Sodium (mmol/L)	14	139.7	137.0-143.0	0	140.0	137.0-142.0	0	140.4	138.0-142.0	0
	21	140.3	138.0-143.0	0	140.0	138.0-145.0	0	139.8	138.0-143.0	0
	28	NE	NE	NE	NE	NE	NE	NE	NE	NE
	0	3.9	3.5-4.3	0	4.1	3.7-4.4	0	4.1	3.7-4.6	0
	7	4.1	3.8-4.6	0	4.1	3.6-4.5	0	4.0	3.6-4.1	0
Potassium (mmol/L)	14	4.0	3.5-4.5	0	4.1	3.7-4.9	0	4.1	3.8-4.3	0
	21	4.1	3.5-4.9	0	4.1	3.7-4.6	0	4.0	3.6-4.4	0
	28	NE	NE	NE	NE	NE	NE	NE	NE	NE

*Not evaluated

Table S4. Laboratory assessment of the participants aged 51-75 years in Phase I

Test (Unit)	Day	5µg group			Placebo group		
		Mean	Range of values	Abnormal values	Mean	Range of values	Abnormal values
Hematocrit (g/dL)	0	43.9	33.0-50.3	0	46.0	43.1-54.6	0
	7	42.5	39.5-50.7	0	45.6	42.1-50.4	0
	14	43.5	35.6-50.6	0	42.5	42.0-50.1	0
	21	43.0	37.2-48.9	0	44.8	41.1-50.2	0
	28	43.1	32.8-50.2	0	44.2	41.1-50.3	0
Hemoglobin (g/dL)	0	13.0	14.1-18.0	0	15.0	14.1-18.0	0
	7	14.0	11.1-17.4	2	15.1	13.8-16.8	0
	14	15.0	11.0-17.1	1	14.0	13.8-16.5	0
	21	14.0	10.1-16.6	1	14.0	13.6-16.6	0
	28	14.0	10.2-16.8	1	14.0	13.6-16.5	0
White Blood Cells (/µliter)	0	6159.6	4300.0-9130.0	0	6692.9	3940.0-9360.0	0
	7	6110.0	4350.0-9050.0	0	6577.5	5090.0-9030.0	0
	14	6212.4	4010.0-9930.0	0	6204.3	3630.0-7640.0	0
	21	6235.0	5080.0-8210.0	0	6290.0	3490.0-7990.0	1
	28	6470.0	3860.0-9430.0	0	6621.7	3980.0-8870.0	0
Neutrophils (%)	0	56.7	41.6-66.3	0	56.8	37.9-66.8	0
	7	53.9	40.1-65.5	0	61.4	40.9-76.0	0
	14	59.4	46.4-73.0	0	50.0	37.0-74.3	0
	21	58.6	41.2-63.0	0	52.7	38.5-70.7	0
	28	56.5	45.8-65.0	0	49.8	38.0-66.0	0
Lymphocytes (%)	0	34.3	25.0-48.0	0	34.4	22.0-56.0	0
	7	37.0	26.0-45.0	0	32.1	19.0-48.0	0
	14	34.8	22.0-44.0	0	35.1	28.0-55.0	0
	21	34.3	6.0-46.0	0	35.9	23.0-52.0	0
	28	35.0	17.0-41.0	0	40.0	26.0-56.0	0
Monocytes (%)	0	6.3	4.0-9.5	0	5.3	3.0-9.4	0
	7	6.1	3.0-9.0	0	6.3	3.9-7.7	0
	14	6.0	3.0-11.0	0	5.8	2.7-9.0	0
	21	6.2	4.0-8.7	0	6.0	3.7-9.9	0
	28	5.2	2.0-8.8	0	5.6	4.0-8.0	0
Eosinophils (%)	0	3.8	0.8-9.0	0	3.2	0.8-8.0	0
	7	3.1	0.9-12.0	0	6.0	2.7-10.0	0
	14	2.8	0.7-10.0	0	4.7	0.7-5.2	0
	21	4.1	1.0-15.0	0	4.3	0.9-5.4	0
	28	3.5	1.0-14.0	0	4.0	1.0-7.0	0
Basophils (%)	0	1.0	0.4-2.3	0	1.0	0.6-1.0	0
	7	2.0	0.2-1.4	0	0.7	0.6-0.9	0
	14	1.0	0.6-1.5	·	0.9	0.6-1.6	0
	21	1.0	0.5-1.2	0	0.7	0.3-1.1	0
	28	1.0	0.3-1.3	0	0.7	0.6-0.7	0
Platelet count (/µliter)	0	265045.5	204000.0-425000.0	0	264125.0	205000.0-318000.0	0
	7	277434.8	195000.0-381000.0	0	267857.1	58000.0-323000.0	1
	14	275428.6	153000.0-384000.0	0	273571.4	60000.0-322000.0	1
	21	279190.5	226000.0-386000.0	0	270500.0	21000.0-312000.0	0

	28	280476.2	183000.0-378000.0	0	277428.6	203000.0-323000.0	0
	0	23.3	16.0-36.0	0	24.8	18.0-35.0	0
	7	23.4	16.0-36.0	0	27.8	21.0-35.0	0
SGOT (U/µliter)	14	21.7	16.0-32.0	0	23.3	19.0-33.0	0
	21	21.8	16.0-43.0	0	20.0	17.0-24.0	0
	28	NE*	NE	NE	NE	NE	NE
	0	19.0	8.0-40.0	0	22.1	13.0-39.0	0
	7	19.0	8.0-47.0	1	21.3	11.0-50.0	2
SGPT (U/µliter)	14	18.6	7.0-30.0	0	22.6	13.0-37.0	0
	21	18.4	10.0-42.0	0	17.8	10.0-31.0	0
	28	NE	NE	NE	NE	NE	NE
	0	165.3	73.0-383.0	1	163.1	101.0-359.0	1
	7	174.7	73.0-366.0	2	197.6	136.0-295.0	0
Alkaline phosphatase (U/µliter)	14	178.5	72.0-316.0	1	171.7	126.0-307.0	1
	21	183.2	88.0-392.0	1	167.4	133.0-320.0	1
	28	NE	NE	NE	NE	NE	NE
	0	0.6	0.4-1.4	1	0.5	0.3-0.7	0
	7	0.6	0.4-1.3	1	0.7	0.5-0.9	0
Bilirubin (mg/dL)	14	0.5	0.1-1.1	0	0.6	0.1-0.9	0
	21	1.0	0.1-1.0	0	0.5	0.2-1.1	0
	28	NE	NE	NE	NE	NE	NE
	0	32.0	22.0-52.0	0	29.5	20.0-49.0	0
	7	29.5	22.0-44.0	0	30.1	22.0-42.0	0
Urea (mg/dL)	14	28.2	17.0-43.0	0	27.9	18.0-47.0	0
	21	32.2	21.0-46.0	0	31.6	24.0-49.0	0
	28	NE	NE	NE	NE	NE	NE
	0	1.0	0.7-1.1	0	1.0	0.7-1.1	0
	7	1.0	0.7-1.2	0	1.0	0.7-1.0	0
Creatinine (mg/dl)	14	1.0	0.7-1.2	0	1.0	0.7-1.0	0
	21	1.0	0.7-1.2	0	0.9	0.7-1.2	0
	28	NE	NE	NE	NE	NE	NE
	0	140.6	138.0-143.0	0	140.3	139.0-141.0	0
	7	140.5	137.0-143.0	0	140.6	138.0-143.0	0
Sodium (mmol/L)	14	141.4	139.0-142.0	0	141.3	138.0-143.0	0
	21	140.6	137.0-143.0	0	140.9	139.0-143.0	0
	28	NE	NE	NE	NE	NE	NE
	0	4.2	3.7-4.6	0	4.0	3.7-4.6	0
	7	4.0	3.5-4.8	0	4.0	3.5-4.2	0
Potassium (mmol/L)	14	4.0	3.9-4.5	0	4.0	3.8-4.6	0
	21	4.0	3.8-4.6	0	4.0	4.0-4.4	0
	28	NE	NE	NE	NE	NE	NE

*Not evaluated

Note: The following laboratory parameters were assessed during the study among all 88 participants of both stages in Phase I at days 0, 7, 14, 21 and 28 after vaccination. As many as 44 participants had abnormal laboratory values; however, none of them were clinically significant. On day 7 after the first injection, a 56-year-old man in the placebo group was presented with thrombocytopenia, which was not severe according to the guidelines of Food and Drug Administration (Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials). He didn't have any clinical signs or symptoms and his platelets returned to normal levels without any actions in the next timepoint.

Table S5. Geometric mean titres, geometric mean ratios, and seroconversion rates of anti-spike IgG (EuroImmun) at different time points in Phase I and Phase II

Antibody	Geometric mean titer (95% CI)			Geometric mean ratio (95% CI)		Seroconversion rate* (95% CI)		
	3µg	5µg	Placebo	3µg	5µg	3µg	5µg	Placebo
Phase I: Stage I								
Day 0	0.32 (0.20-0.51)	0.19 (0.13-0.27)	0.12 (0.09-0.18)	2.57 (1.12-5.9)	1.51 (0.79-2.88)	N/A**	N/A	N/A
Day 14	0.69 (0.35-1.36)	0.40 (0.24-0.65)	0.12 (0.09-0.18)	5.49 (1.67-18.1)	3.19 (1.34-7.61)	29.17 (12.62-51.09)	33.33 (15.63-55.32)	0 (0-0)
Day 21	1.30 (0.67-2.50)	4.44 (3.10-6.36)	0.20 (0.15-0.27)	6.39 (2.04-19.98)	21.89 (11.58-41.36)	41.67 (22.11-63.36)	91.67 (73.00-98.97)	0 (0-0)
Day 28	1.26 (0.65-2.41)	4.37 (3.43-5.57)	0.13 (0.10-0.17)	9.68 (3.11-30.09)	33.68 (21.62-52.47)	41.67 (22.11-63.36)	91.67 (73.00-98.97)	0 (0-0)
Phase I: Stage II								
Day 0	N/A	0.20 (0.12-0.32)	0.13 (0.07-0.21)	N/A	1.56 (0.67-3.63)	N/A	N/A	N/A
Day 14	N/A	0.33 (0.18-0.63)	0.12 (0.08-0.20)	N/A	2.73 (0.93-8.02)	N/A	9.09 (1.12-29.16)	0 (0-0)
Day 21	N/A	1.89 (1.11-3.22)	0.12 (0.08-0.20)	N/A	15.42 (6.18-38.49)	N/A	63.64 (40.66-82.80)	0 (0-0)
Day 28	N/A	3.07 (1.82-5.18)	0.14 (0.08-0.26)	N/A	21.40 (8.59-53.32)	N/A	77.27 (54.63-92.18)	12.5 (0.32-52.65)
Phase II								
Day 0	N/A	0.25 (0.21-0.29)	0.25 (0.18-0.36)	N/A	0.99 (0.69-1.43)	N/A	N/A	N/A
Day 28	N/A	1.42 (1.17-1.73)	0.36 (0.25-0.53)	N/A	3.91 (2.56-5.95)	N/A	63.64 (56.72-70.16)	16.36 (7.77-28.80)
Day 42	N/A	4.52 (3.95-5.17)	0.61 (0.38-0.96)	N/A	7.48 (5.26-10.62)	N/A	83.25 (77.49-88.05)	25.45 (14.67-39.00)

**Not applicable

Note: Anti-spike IgG (EuroImmun antibody) was reported for both Phases. For Phase I, findings were reported at baseline (day 0), two weeks after the first vaccination (day 14), and two weeks after the second vaccination (day 28) for 3 µg, 5 µg and placebo groups. For Phase II, findings were reported at baseline (day 0), four weeks after the first vaccination (day 28), and two weeks after the second vaccination (day 42) for 5µg and placebo groups. In stage I, one participant in the 3 µg group became PCR positive for COVID-19 on day 7th after the first dose and was thus excluded from the study. In stage II, one participant in the 5 µg group was excluded from the study and did not receive any doses due to white coat syndrome. Another participant in the 5 µg group of Stage II became PCR positive for COVID-19 within a day after second injection and thus was excluded from data analysis. In Phase II, 11 participants in the 5µg group were excluded from the study due to positive RT-PCR for COVID-19 after first injection (N=9), death due to suicide via cyanide toxicity (N=1) and co-administration of another COVID-19 vaccine platform without prior notice (N=1).

Table S6. Solicited and unsolicited adverse events among participants aged 18-75 years in Phase II

Adverse events		Solicited AEs				Unsolicited AEs			
		First administration n(%)		Second administration n(%)		First administration n(%)		Second administration n(%)	
		Placebo	5 μg	Placebo	5 μg	Placebo	5 μg	Placebo	5 μg
Injection site involvement	Pain at injection site	9(16.1)	45(20.1)	10(17.9)	33(15.5)	0(0.0)	1(0.4)	0(0.0)	11(5.2)
	Redness at injection site	0(0.0)	1(0.4)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
General reactions	Myalgia	2(3.6)	14(5.4)	2(3.6)	9(4.2)	0(0.0)	2(0.9)	0(0.0)	3(1.4)
	Weakness	0(0.0)	7(3.1)	0(0.0)	1(0.5)	0(0.0)	3(1.3)	1(1.8)	4(1.9)
	Fever	2(3.6)	7(3.1)	1(1.8)	2(0.9)	0(0.0)	1(0.4)	0(0.0)	2(0.9)
	Chills	0(0.0)	1(0.4)	0(0.0)	1(0.4)	0(0.0)	0(0.0)	1(1.8)	3(1.4)
	Fatigue	0(0.0)	0(0.0)	0(0.0)	6(2.8)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
	Flushing	0(0.0)	4(1.8)	1(1.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Increased sweating	1(1.8)	2(0.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Skeletal pain	0(0.0)	0(0.0)	1(1.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(0.9)
	Sleep disorder	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Upper and lower respiratory system disorders	Sore throat	1(1.8)	3(1.3)	0(0.0)	1(0.5)	0(0.0)	1(0.4)	2(3.6)	5(2.3)
	Coughing	1(1.8)	3(1.3)	0(0.0)	0(0.0)	0(0.0)	2(0.9)	1(1.8)	0(0.0)
	Rhinitis	1(1.8)	2(0.9)	0(0.0)	0(0.0)	0(0.0)	2(0.9)	1(1.8)	0(0.0)
	Dyspnea	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
	Epistaxis	0(0.0)	1(0.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Tachypnea	0(0.0)	0(0.0)	1(1.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Chest pain	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
Gastrointestinal, stomach and urinary disorders	Abdominal pain	0(0.0)	1(0.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(1.4)
	Diarrhea	1(1.8)	2(0.9)	2(3.6)	3(1.4)	0(0.0)	1(0.4)	1(1.8)	2(0.9)
	Nausea	0(0.0)	1(0.4)	0(0.0)	2(0.9)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
	Vomiting	0(0.0)	1(0.4)	0(0.0)	3(1.4)	0(0.0)	0(0.0)	1(1.8)	0(0.0)
Skin and subcutaneous tissue disorders	Pruritus	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Skin rashes	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1 (1.8)	0(0.0)
	Acne	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1 (1.8)	0(0.0)
Cardiovascular system disorders	Hypotension (systolic)	0(0.0)	1(0.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Hypertension (systolic)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Tachycardia	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1 (0.4)	0(0.0)	0(0.0)
Nervous system disorders	Dizziness	0(0.0)	1(0.4)	0(0.0)	1(0.5)	0(0.0)	1(0.4)	1(1.8)	1(0.5)
	Headache	1(1.8)	12(5.4)	7(3.3)	7(3.3)	1(1.8)	2(0.9)	2(3.6)	7(3.3)
	Dysphonia	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	0(0.0)	0(0.0)
Infections and infestations	Fungal dermatitis	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
	Dental Abscess	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1 (0.4)	0(0.0)	0(0.0)

Table S7. Geometric mean titres, geometric mean ratios, and seroconversion rates of neutralising antibody at different time points in Phase I and Phase II after sensitivity analysis

Antibody	Geometric mean titer (95% CI)			Geometric mean ratio (95% CI)		Seroconversion rate* (95% CI)		
	3µg	5µg	Placebo	3µg	5µg	3µg	5µg	Placebo
Phase I: Stage I								
Day 0	1.43 (1.22-1.68)	1.19 (0.74-1.92)	1.55 (1.20-2.01)	0.92 (0.69-1.23)	0.76 (0.35-1.67)	N/A**	N/A	N/A
Day 14	1.51 (0.93-2.45)	2.09 (1.26-3.48)	2.36 (0.70-8.00)	0.64 (0.23-1.73)	0.89 (0.31-2.49)	0 (0-0)	19.05 (5.45-41.91)	12.5 (0.32-52.65)
Day 21	4.80 (2.46-9.34)	6.32 (2.79-14.29)	1.31 (1.08-1.60)	3.66 (1.28-10.48)	4.82 (1.29-18.03)	25.00 (8.66-49.10)	57.14 (34.02-78.18)	0. (0-0)
Day 28	4.97 (2.26-10.89)	14.75 (7.43-29.26)	2.76 (0.63-12.11)	1.80 (0.42-7.79)	5.35 (1.39-20.54)	35.00 (15.39-59.22)	71.43 (47.82-88.72)	37.5 (8.52-75.51)
Phase I: Stage II								
Day 0	N/A	0.37 (0.28-0.48)	0.56 (0.41-0.76)	N/A	0.65 (0.41-1.04)	N/A	N/A	N/A
Day 14	N/A	0.92 (0.42-2.02)	0.31 (0.20-0.49)	N/A	2.93 (0.78-10.97)	N/A	22.73 (7.82-45.37)	0 (0-0)
Day 21	N/A	5.39 (2.69-10.83)	0.80 (0.46-1.40)	N/A	6.70 (2.05-21.94)	N/A	77.27 (54.63-92.18)	12.5 (0.32-52.65)
Day 28	N/A	12.52 (7.29-21.51)	0.85 (0.27-2.66)	N/A	14.81 (5.11-42.93)	N/A	100 (84.56-100)	12.5 (0.32-52.65)
Phase II								
Day 0	N/A	0.23 (0.20-0.26)	0.28 (0.21-0.38)	N/A	0.80 (0.60-1.08)	N/A	N/A	N/A
Day 28	N/A	1.16 (0.85-1.57)	0.30 (0.19-0.47)	N/A	3.92 (2.03-7.54)	N/A	51.24 (44.11-58.34)	14.00 (5.82-26.74)
Day 42	N/A	10.98 (8.32-14.47)	0.62 (0.32-1.18)	N/A	17.76 (9.36-33.71)	N/A	85.07 (79.38-89.70)	28.00 (16.23-42.49)

**Not applicable

Geometric mean titres for neutralising antibody is reported in µg/ml.

Note: For Phase I, findings were reported at baseline (day 0), two weeks after the first vaccination (day 14), and two weeks after the second vaccination (day 28) for 3 µg, 5 µg and placebo groups. For Phase II, findings were reported at baseline (day 0), four weeks after the first vaccination (day 28), and two weeks after the second vaccination (day 42) for 5µg and placebo groups. In stage I, one participant in the 3 µg group became PCR positive for COVID-19 on day 7th after the first dose and was thus excluded from the study. In stage II, one participant in the 5 µg group was excluded from the study and did not receive any doses due to white coat syndrome. Another participant in the 5 µg group of Stage II became PCR positive for COVID-19 within a day after second injection and thus was excluded from data analysis. In Phase II, 11 participants in the 5µg group were excluded from the study due to positive RT-PCR for COVID-19 after first injection (N=9), death due to suicide via cyanide toxicity (N=1) and co-administration of another COVID-19 vaccine platform without prior notice (N=1).

Table S8. Geometric mean titres, geometric mean ratios, and seroconversion rates of anti-receptor binding IgG at different time points in Phase I and Phase II after sensitivity analysis

Antibody	Geometric mean titer (95% CI)			Geometric mean ratio (95% CI)		Seroconversion rate* (95% CI)		
	3µg	5µg	Placebo	3µg	5µg	3µg	5µg	Placebo
Phase I: Stage I								
Day 0	0.10 (0.10-0.10)	0.14 (0.08-0.23)	0.10 (0.10-0.10)	1.00 (1.00, 1.00)	1.38 (0.60, 3.13)	N/A**	N/A	N/A
Day 14	0.21 (0.10-0.44)	2.24 (1.18-4.29)	0.10 (0.10-0.10)	2.11 (0.67, 6.64)	22.44 (7.90, 63.73)	20.00 (5.73, 43.66)	80.95 (58.09, 94.55)	0 (0, 0)
Day 21	0.58 (0.24-1.39)	8.38 (5.65-12.43)	0.14 (0.08-0.28)	4.04 (0.98, 16.58)	58.39 (28.42, 119.98)	20.00 (5.73, 43.66)	95.24 (76.18, 99.88)	12.5 (0.32, 52.65)
Day 28	0.86 (0.37-2.01)	8.12 (6.05-10.89)	0.12 (0.09-0.16)	7.25 (1.9, 27.67)	68.27 (41.67, 111.87)	65.00 (40.78, 84.61)	95.24 (76.18, 99.88)	0 (0, 0)
Phase I: Stage II								
Day 0	N/A	0.14 (0.10-0.21)	0.10 (0.10-0.10)	N/A	1.40 (0.74, 2.65)	N/A	N/A	N/A
Day 14	N/A	0.30 (0.14-0.66)	0.10 (0.10-0.10)	N/A	3.03 (0.84, 10.86)	N/A	22.73 (7.82, 45.37)	0 (0, 0)
Day 21	N/A	4.00 (1.84-8.71)	0.10 (0.10-0.10)	N/A	39.98 (11.05, 144.58)	N/A	77.27 (54.63, 92.18)	0 (0, 0)
Day 28	N/A	6.02 (3.26-11.13)	0.10 (0.10-0.10)	N/A	60.23 (21.83, 166.16)	N/A	86.36 (65.09, 97.09)	0 (0, 0)
Phase II								
Day 0	N/A	0.21 (0.18-0.25)	0.19 (0.14-0.25)	N/A	1.15 (0.82, 1.61)	N/A	N/A	N/A
Day 28	N/A	0.98 (0.76-1.27)	0.26 (0.17-0.42)	N/A	3.74 (2.14, 6.54)	N/A	51.24 (44.11, 58.34)	20.00 (10.03, 33.72)
Day 42	N/A	2.86 (2.37-3.44)	0.38 (0.23-0.64)	N/A	7.44 (4.75, 11.65)	N/A	77.61 (71.21, 83.18)	30.00 (17.86, 44.61)

*,**Not applicable

Geometric mean titres for anti-receptor binding domain IgG is reported in RU/ml.

Note: For Phase I, findings were reported at baseline (day 0), two weeks after the first vaccination (day 14), and two weeks after the second vaccination (day 28) for 3 µg, 5 µg and placebo groups. For Phase II, findings were reported at baseline (day 0), four weeks after the first vaccination (day 28), and two weeks after the second vaccination (day 42) for 5µg and placebo groups. In stage I, one participant in the 3 µg group became PCR positive for COVID-19 on day 7th after the first dose and was thus excluded from the study. In stage II, one participant in the 5 µg group was excluded from the study and did not receive any doses due to white coat syndrome. Another participant in the 5 µg group of Stage II became PCR positive for COVID-19 within a day after second injection and thus was excluded from data analysis. In Phase II, 11 participants in the 5µg group were excluded from the study due to positive RT-PCR for COVID-19 after first injection (N=9), death due to suicide via cyanide toxicity (N=1) and co-administration of another COVID-19 vaccine platform without prior notice (N=1).

Table S9. Geometric mean titres, geometric mean ratios, and seroconversion rates of anti-spike glycoprotein IgG at different time points in Phase I and Phase II after sensitivity analysis

Antibody	Geometric mean titer (95% CI)			Geometric mean ratio (95% CI)		Seroconversion rate* (95% CI)		
	3µg	5µg	Placebo	3µg	5µg	3µg	5µg	Placebo
Phase I: Stage I								
Day 0	0.40 (0.16-0.97)	0.16 (0.10-0.25)	0.11 (0.09-0.13)	3.71 (0.91, 15.04)	1.51 (0.74, 3.08)	N/A**	N/A	N/A
Day 14	1.17 (0.34-4.07)	1.60 (0.62-4.08)	0.19 (0.08-0.46)	6.08 (0.81, 45.45)	8.28 (1.71, 40.17)	30.00 (11.89, 54.28)	71.43 (47.82, 88.72)	12.5 (0.32, 52.65)
Day 21	6.52 (2.75-15.46)	50.89 (31.94-81.08)	0.68 (0.30-1.55	9.55 (2.30, 39.68)	74.53 (31.47, 176.52)	80.00 (56.34, 94.27)	100 (83.89, 100)	75.00 (34.91, 96.81)
Day 28	6.72 (2.50-18.02)	67.26 (50.90-88.89)	0.30 (0.13-0.73)	22.15 (4.38, 111.94)	221.87 (116.74, 421.67)	80.00 (56.34, 94.27)	100 (83.89, 100)	50.00 (15.70, 84.30)
Phase I: Stage II								
Day 0	N/A	0.33 (0.14-0.75)	0.27 (0.11-0.67)	N/A	1.23 (0.29, 5.18)	N/A	N/A	N/A
Day 14	N/A	0.69 (0.24-1.98)	0.19 (0.10-0.34)	N/A	3.69 (0.63, 21.75)	N/A	18.18 (5.19, 40.28)	0 (0, 0)
Day 21	N/A	19.56 (7.63-50.09)	0.17 (0.09-0.31)	N/A	117.17 (24.04, 571.11)	N/A	72.73 (49.78, 89.27)	0 (0, 0)
Day 28	N/A	53.69 (29.09-99.10)	0.18 (0.09-0.40)	N/A	292.79 (98.91, 866.70)	N/A	86.36 (65.09, 97.09)	12.5 (0.32, 52.65)
Phase II								
Day 0	N/A	0.51 (0.38-0.68)	0.33 (0.19-0.55)	N/A	1.55 (0.82, 2.94)	N/A	N/A	N/A
Day 28	N/A	8.19 (5.89-11.39)	0.64 (0.34-1.22)	N/A	12.73 (6.14, 26.42)	N/A	70.15 (63.31, 76.38)	24.00 (13.06, 38.17)
Day 42	N/A	37.12 (28.86-47.76)	2.98 (1.29-6.86)	N/A	12.46 (6.51, 23.82)	N/A	82.09 (76.08, 87.13)	48.00 (33.66, 62.58)

*,**Not applicable

Geometric mean titres for anti-spike glycoprotein IgG RU/ml is reported in RU/ml.

Note: For Phase I, findings were reported at baseline (day 0), two weeks after the first vaccination (day 14), and two weeks after the second vaccination (day 28) for 3 µg, 5 µg and placebo groups. For Phase II, findings were reported at baseline (day 0), four weeks after the first vaccination (day 28), and two weeks after the second vaccination (day 42) for 5µg and placebo groups. In stage I, one participant in the 3 µg group became PCR positive for COVID-19 on day 7th after the first dose and was thus excluded from the study. In stage II, one participant in the 5 µg group was excluded from the study and did not receive any doses due to white coat syndrome. Another participant in the 5 µg group of Stage II became PCR positive for COVID-19 within a day after second injection and thus was excluded from data analysis. In Phase II, 11 participants in the 5µg group were excluded from the study due to positive RT-PCR for COVID-19 after first injection (N=9), death due to suicide via cyanide toxicity (N=1) and co-administration of another COVID-19 vaccine platform without prior notice (N=1).

بررسی ایمنی و ایمنی‌زایی واکسن حاوی آنتی‌ژن ویروس غیرفعال‌شده‌ی BIV1-CovIran (کووایران) در فازهای یک و دو مطالعات بالینی: مطالعات دوسوکور، تصادفی و کنترل‌شده با واکسن‌نما

مقدمه

تلاش فراوانی در سطح جهانی برای تولید سریع واکسن‌های ایمن و موثر در برابر ویروس عامل سندرم حاد تنفسی کرونا ۲ (SARS-CoV-2) و کنترل همه‌گیری بیماری ویروس کرونا ۲۰۱۹ (COVID-19) انجام شده است. این مطالعه، نتایج فازهای اول و دوم کارآزمایی‌های بالینی واکسن BIV1-CovIran (کووایران) حاوی آنتی‌ژن ویروس غیرفعال‌شده را با هدف ارزیابی ایمنی و ایمنی‌زایی آن گزارش می‌کند.

مواد و روش‌ها

این کارآزمایی‌های بالینی به‌صورت دوسوکور، تصادفی و کنترل‌شده با واکسن‌نما برای ارزیابی ایمنی و ایمنی‌زایی BIV1-CovIran (کووایران) انجام شده‌اند. داوطلبانی با نتیجه‌ی منفی تست واکنش زنجیره‌ای پلیمراز (RT-PCR) و آزمایش‌های سرولوژی برای SARS-CoV-2 برای شرکت در این کارآزمایی‌های بالینی ثبت‌نام کردند. فاز یک کارآزمایی بالینی در دو مرحله انجام شد. مرحله اول با مشارکت افراد سنین ۱۸-۵۰ سال و مرحله دوم با مشارکت افراد سنین ۵۱-۷۵ سال اجرا گردید. در مرحله اول، شرکت‌کنندگان به طور تصادفی به سه گروه با نسبت های سه، سه و یک تخصیص یافتند و به ترتیب واکسن‌های حاوی سه میکروگرم آنتی‌ژن ویروس غیرفعال‌شده، واکسن حاوی پنج میکروگرم آنتی‌ژن ویروس غیرفعال‌شده و یا واکسن‌نما دریافت کردند. فاصله‌ی دو تزریق در هر سه گروه در مرحله‌ی اول فاز یک، ۱۴ روز بود. بر اساس یافته‌های ایمنی‌زایی مرحله‌ی اول، شرکت‌کنندگان در مرحله‌ی دوم به دو گروه با نسبت‌های چهار و یک تخصیص یافتند و به ترتیب واکسن حاوی پنج میکروگرم آنتی‌ژن ویروس غیرفعال‌شده و یا واکسن‌نما دریافت کردند. فاصله دو تزریق در هر دو گروه در مرحله‌ی دوم فاز یک ۱۴ روز بود. در فاز دو، شرکت‌کنندگان مطالعه به دو گروه با نسبت‌های سه و یک تخصیص یافتند و به ترتیب واکسن حاوی پنج میکروگرم آنتی‌ژن ویروس غیرفعال‌شده و یا واکسن‌نما دریافت کردند. با توجه به نتایج فاز یک و نیز یافته‌های مطالعات بالینی روی سایر واکسن‌ها، فاصله‌ی دو تزریق در هر دو گروه در فاز دو به ۲۸ روز افزایش یافت. شرکت‌کنندگان مطالعه، ارزیابی‌کنندگان نتایج، متخصصان آمار و سایر پرسنل مرتبط با مطالعه هیچ‌گونه اطلاعی از گروه‌بندی هر شرکت‌کننده در مطالعه نداشتند. ایمنی واکسن و قابلیت ایمنی‌زایی آن در هر دو فاز کارآزمایی بررسی گردید. میانگین هندسی تیتر پادتن‌ها (GMT)، نسبت‌های میانگین هندسی تیتر پادتن‌ها در گروه

مداخله به گروه واکسن‌نما (GMR) و نیز seroconversion rate برای پادتن‌های تولید شده علیه SARS-CoV-2 شامل neutralising antibody، anti-RBD IgG و نیز anti-spike glycoprotein IgG گزارش شد. همچنین، نتایج Conventional virus neutralization test گزارش شد.

یافته‌ها

مرحله‌ی اول فاز یک کارآزمایی بالینی با مشارکت ۵۶ نفر انجام شد که از این میان، ۲۴ نفر در گروه سه میکروگرم، ۲۴ نفر در گروه پنج میکروگرم و ۸ نفر در گروه واکسن‌نما قرار داشتند. میانگین (انحراف معیار) سن شرکت‌کنندگان در مرحله‌ی اول فاز یک، به ترتیب ۳۴/۰(۸/۶)، ۳۵/۰(۶/۸) و ۳۴/۴(۷/۸) بود. میانگین (انحراف معیار) سن شرکت‌کنندگان در مرحله‌ی دوم فاز یک، ۵۸/۵(۶/۹) در گروه پنج میکروگرم و ۵۵/۵(۳/۵) در گروه واکسن‌نما بود. بروز کلی رخدادهای نامطلوب تا هفت روز پس از اولین تزریق، معادل ۱۴(۵۸/۳٪) در گروه سه میکروگرم، ۱۶(۶۶/۷٪) در گروه پنج میکروگرم و ۶(۷۵/۰٪) در گروه واکسن‌نما بود. بروز کلی رخدادهای نامطلوب تا هفت روز پس از دومین تزریق، معادل ۱۴(۶۰/۹٪) در گروه سه میکروگرم، ۱۸(۷۵/۰٪) در گروه پنج میکروگرم و ۶(۷۵/۰٪) در گروه واکسن‌نما بود. در میان شرکت‌کنندگان گروه پنج میکروگرم در مرحله‌ی دوم فاز یک کارآزمایی بالینی، ۱۵(۴۸/۴٪) نفر تا هفت روز پس از اولین تزریق و ۱۸(۵۸/۱٪) نفر تا هفت روز پس از دومین تزریق حداقل بروز یک رخداد نامطلوب را گزارش کردند. در فاز یک کارآزمایی بالینی، شدت همه‌ی رخدادهای نامطلوب خفیف یا متوسط بوده و هیچگونه adverse events of special interest گزارش نشد. در بررسی‌های ایمنی‌زایی واکسن در مرحله‌ی اول فاز یک، seroconversion rate (فاصله اطمینان ۹۵٪) برای neutralising antibody ، anti-RBD IgG و anti-spike glycoprotein IgG در روز چهاردهم پس از دومین تزریق واکسن پنج میکروگرم در مرحله اول فاز یک کارآزمایی بالینی به ترتیب معادل ۷۰/۸٪(۴۸/۹-۸۷/۴)، ۸۷/۵٪(۶۷/۶-۹۷/۳) و ۹۱/۷٪(۷۳/۰-۹۹/۰) بوده‌است. این مقادیر در گروهی از شرکت‌کنندگان که واکسن سه میکروگرم را در مرحله اول فاز یک کارآزمایی بالینی دریافت کرده‌بودند به ترتیب معادل ۴۵/۸٪(۲۵/۶-۶۷/۲)، ۵۴/۲٪(۳۲/۸-۷۴/۵) و ۷۰/۸٪(۴۸/۹-۸۷/۴) گزارش گردید. در بررسی‌های ایمنی‌زایی واکسن در مرحله‌ی دوم فاز یک، seroconversion rate (فاصله اطمینان ۹۵٪) برای neutralising antibody ، anti-RBD IgG و anti-spike glycoprotein IgG در روز چهاردهم پس از دومین تزریق واکسن معادل ۱۰۰/۰٪(۸۴/۶-۱۰۰/۰)، ۸۶/۴٪(۶۵/۱-۹۷/۱) و ۸۶/۴٪(۶۵/۱-۹۷/۱) بود.

فاز دو کارآزمایی بالینی با مشارکت ۲۸۰ نفر انجام شد که از میان آن‌ها، ۲۲۴ نفر در گروه پنج میکروگرم و ۵۶ نفر در گروه واکسن‌نما قرار داشتند. میانگین (انحراف معیار) سن شرکت‌کنندگان در گروه مداخله و واکسن‌نما، به ترتیب ۴۲/۲(۱۲/۸) و ۴۰/۴(۱۲/۴) بود. در گروه پنج میکروگرم، بروز کلی رخدادهای نامطلوب (۶۸(۳۰/۴٪ تا هفت روز پس از اولین تزریق و (۵۴(۲۵/۳٪ تا هفت روز پس از دومین تزریق بود. این میزان در گروه واکسن‌نما به ترتیب (۱۲(۲۱/۴٪ و (۱۸(۳۲/۱٪ بود. همانند فاز یک، در فاز دو نیز، هیچگونه adverse events of special interest گزارش نشد. GMT همه‌ی پادتن‌ها پس از تزریق دو دوز واکسن افزایش یافت. در روز ۴۲، seroconversion rate (فاصله اطمینان ۹۵٪) neutralising antibody معادل (۸۷/۶-۷۷/۰)۸۲/۸٪، anti-RBD IgG معادل (۸۲/۶-۷۰/۷)۷۷/۰٪ و برای anti-spike glycoprotein IgG (۸۵/۱-۷۳/۸)۷۹/۹٪ بود.

نتیجه‌گیری

ارزیابی ایمنی و اثربخشی تزریق دو نوبت واکسن BIV1-CovIran (کووایران) حاوی پنج میکروگرم ذرات غیرفعال شده‌ی ویروس SARS-CoV-2 با فاصله ۲۸ روز در مطالعات کارآزمایی بالینی فاز سه ضروری است.

منابع مالی

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