BMJ Open

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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To cite: 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

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2021-056872).

Received 30 August 2021
Accepted 16 March 2022

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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

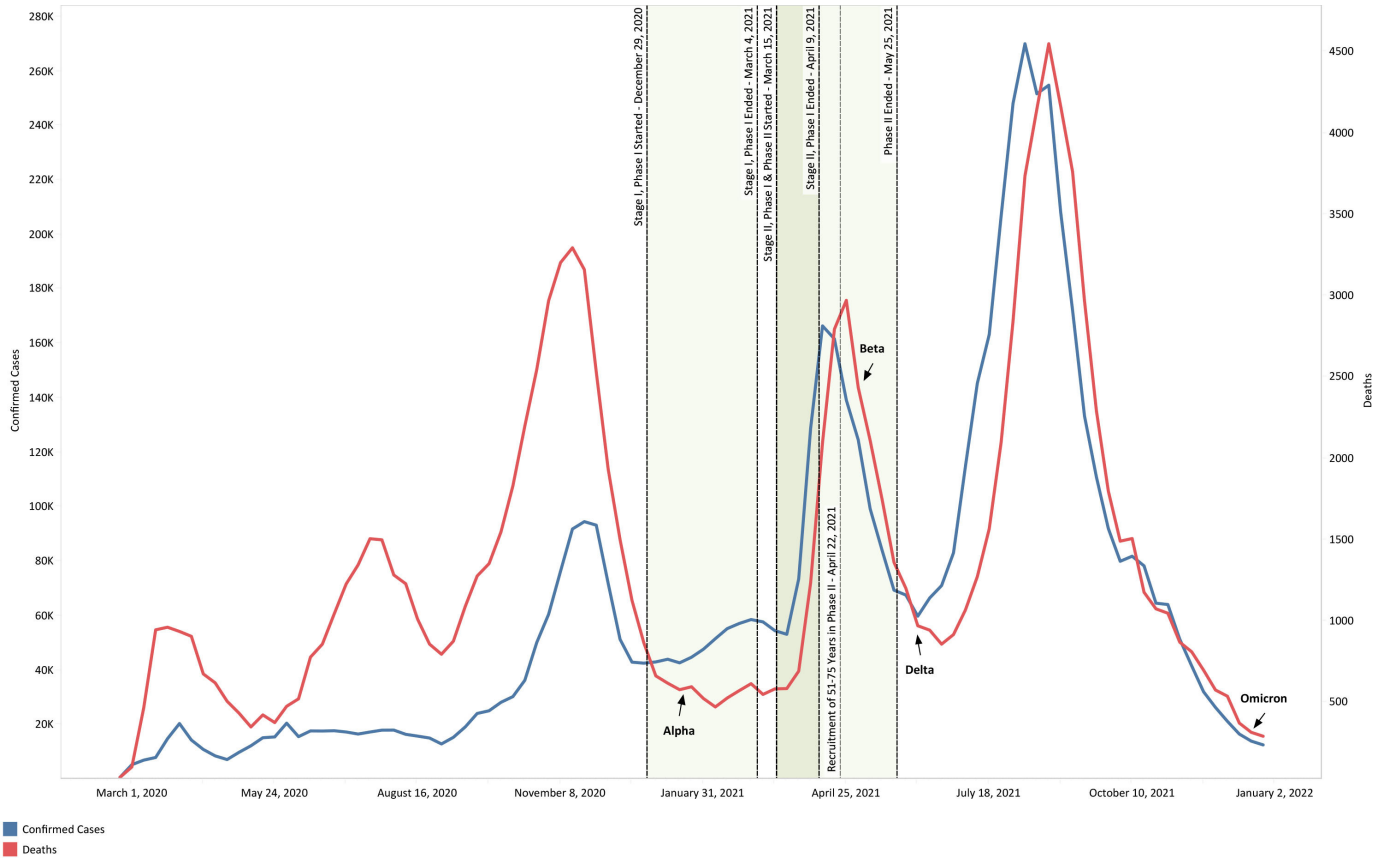

Figure 1 Mapping the timeline of phase I and phase II clinical trials with the time trend of COVID-19 weekly new cases (blue line) and mortality (red line) in Iran by the first week of January 2022.

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

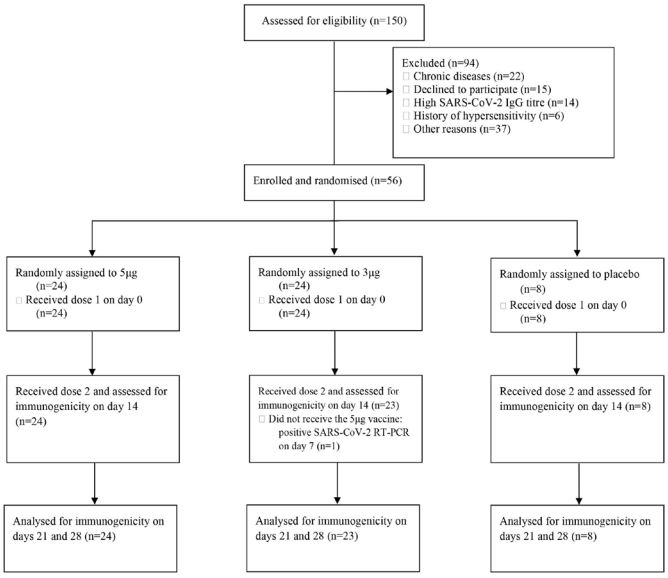

Figure 2 Diagram of screening, enrolment, randomisation and follow-up in stage I of phase I. RT-PCR, reverse transcription PCR.

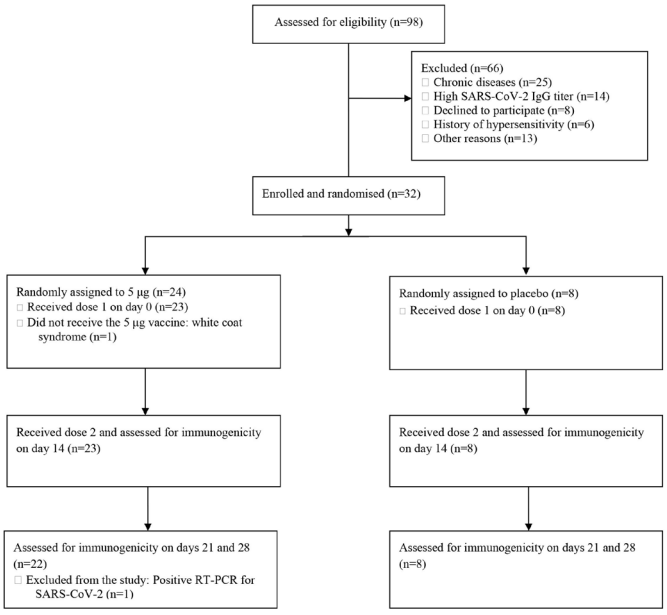

Figure 3 Diagram of screening, enrolment, randomisation and follow-up in stage II of phase I. RT-PCR, reverse transcription PCR.

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

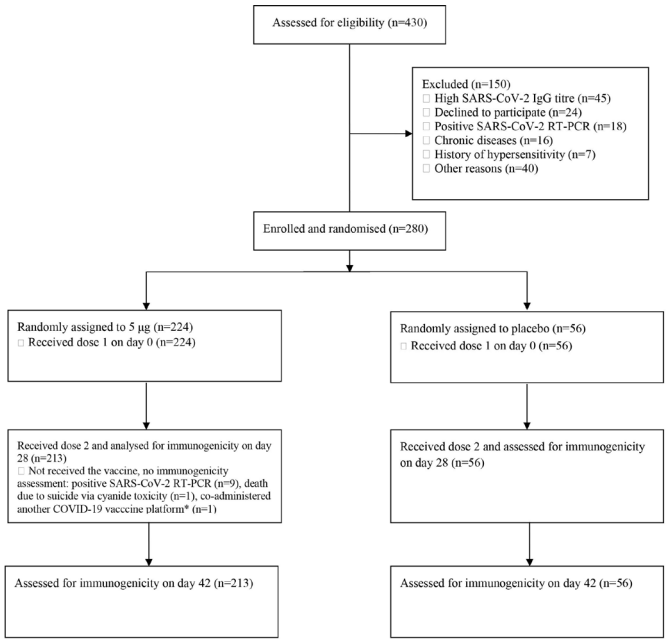

Figure 4 Diagram of screening, enrolment, randomisation and follow-up in phase II. *The latter participant reported administering a dose of another vaccine after the first injection of BIV1-CovIran, and was thus excluded from the study. RT-PCR, reverse transcription PCR.

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.

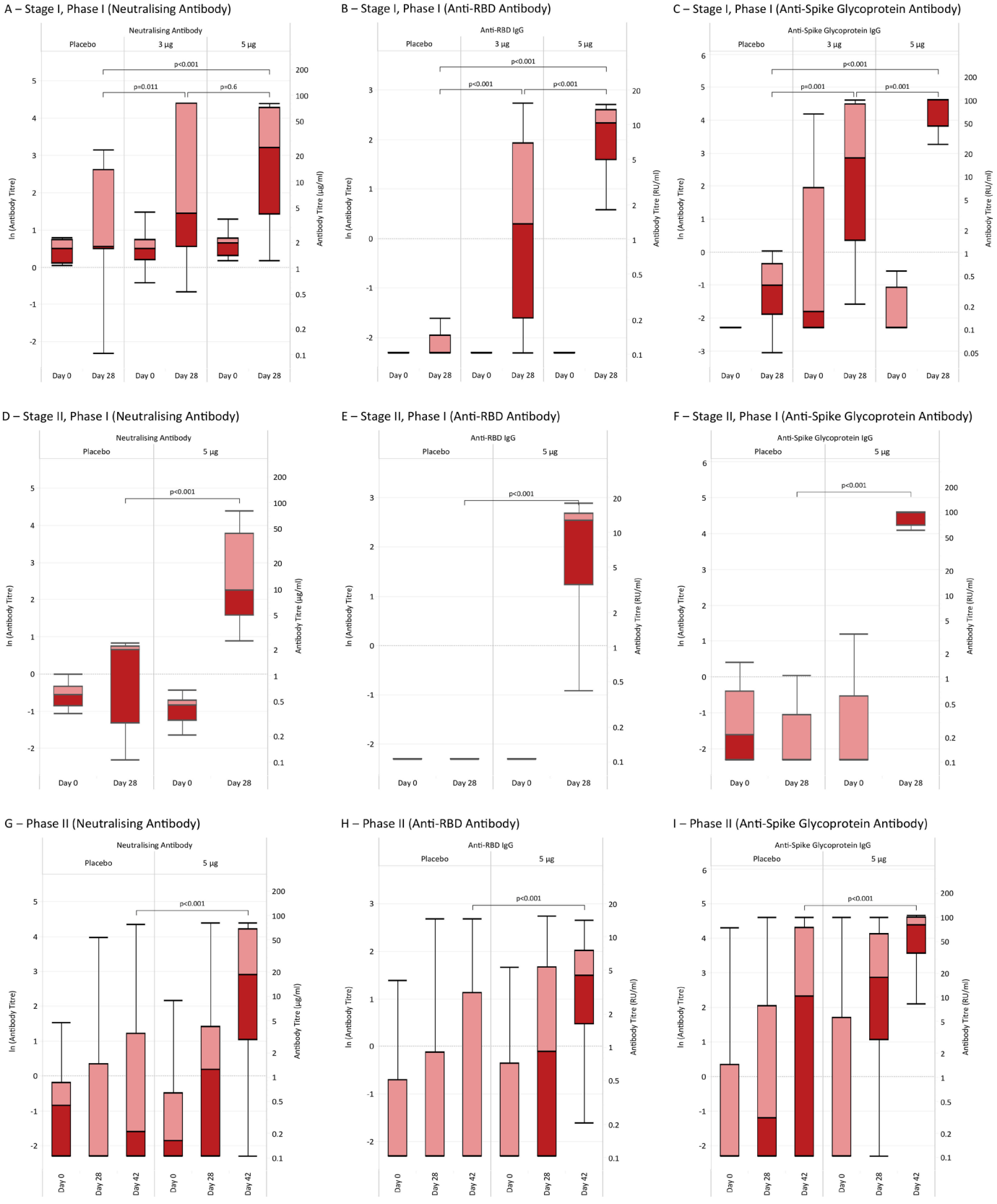

Figure 5 Anti-SARS-CoV-2 antibody titres for neutralising, anti-RBD, and anti-spike glycoprotein antibodies in stage I (A, B, C), stage II (D, E, F) and phase II (G, H, I). Box plots present second quartile in dark red and third quartile in pale red. RBD, receptor binding-domain.

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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Acknowledgements This work was supported and funded by Shifa Pharmed Industrial Group. The funding source provided vaccine and placebo vials and other necessary equipment and trial site preparation. The trial sponsor had no role in collecting and interpreting data, manuscript preparation, and the decision for submission. The authors would like to thank the staff of Clinical Trial Center, Tehran University of Medical Sciences, Tehran, Iran for their constant support in the clinical trial management and monitoring. The authors thank Professor Hamidreza Jamshidi, Professor Farshad Farzadfar, Professor Hassan Jalili, Dr. Akbar Borandegi, Dr. Mohammad Taqavian, Dr. Asghar Abdoli, Professor Farshid Noorbakhsh, Dr. Nazila Rezaei, and Dr. Mohammad-Mahdi Rashidi for their invaluable supports. Masked laboratory assessments were done at Noor Pathobiology and Genetic laboratory, and masked datasheets were sent to the third-party research centre for decoding and analysis. The authors would like to thank all participants of the study. They would also like to express their most sincere gratitude to all frontline healthcare workers across the globe during this pandemic.

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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REFERENCES

1 Forni G, Mantovani A, Forni G, COVID-19 Commission of Accademia Nazionale dei Lincei, Rome. COVID-19 vaccines: where we stand and challenges ahead. *Cell Death Differ* 2021;28:626–39.
2 Isakova-Sivak I, Rudenko L. A promising inactivated whole-virion SARS-CoV-2 vaccine. *Lancet Infect Dis* 2021;21:2–3.
3 Mulligan MJ, Lyke KE, Kitchin N, *et al*. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 2020;586:589–93.
4 COVID-19 vaccine tracker and landscape. Available: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines [Accessed 6 Aug 2021].
5 Kim JH, Marks F, Clemens JD. Looking beyond COVID-19 vaccine phase 3 trials. *Nat Med* 2021;27:205–11.
6 World Health Organization. Draft landscape and tracker of COVID-19 candidate vaccines.
7 World Health Organization. Status of COVID-19 vaccines within WHO EUL/PQ evaluation process.
8 Sharif N, Alzahrani KJ, Ahmed SN, *et al*. Efficacy, immunogenicity and safety of COVID-19 vaccines: a systematic review and meta-analysis. *Front Immunol* 2021;12.
9 Rotshild V, Hirsh-Raccah B, Miskin I, *et al*. Comparing the clinical efficacy of COVID-19 vaccines: a systematic review and network meta-analysis. *Sci Rep* 2021;11:22777.
10 Liu Q, Qin C, Liu M, *et al*. Effectiveness and safety of SARS-CoV-2 vaccine in real-world studies: a systematic review and meta-analysis. *Infect Dis Poverty* 2021;10:132.
11 Feikin DR, Higdon MM, Abu-Raddad LJ, *et al*. Duration of effectiveness of vaccines against SARS-CoV-2 infection and COVID-19 disease: results of a systematic review and meta-regression. *Lancet* 2022;399:924–44.
12 The Lancet Infectious Diseases. The Rocky road to universal COVID-19 vaccination. *Lancet Infect Dis* 2021;21:743.
13 Ritchie H, Ortiz-Ospina E, Beltekian D. Coronavirus pandemic (COVID-19), 2020. Available: https://ourworldindata.org/coronavirus [Accessed 6 Aug 2021].

14 Takian A, Raoofi A, Kazempour-Ardebili S. COVID-19 battle during the toughest sanctions against Iran. *Lancet* 2020;395:1035–6.
15 Statistical Center of Iran. Results of 2016 Iranian population and housing censuses, 2016. Available: https://www.amar.org.ir/Portals/1/census/2016/Population-and-Household-by-Province-and-Shahrestan.xlsx [Accessed 27 Aug 2020].
16 Danaei G, Farzadfar F, Kelishadi R, *et al*. Iran in transition. *Lancet* 2019;393:1984–2005.
17 Abdoli A, Aalizadeh R, Aminianfar H, *et al*. Safety and potency of BIV1-CovIran inactivated vaccine candidate for SARS-CoV-2: a preclinical study. *Rev Med Virol* 2021:e2305.
18 IRCT. A double-blinded, randomized, placebo-controlled phase I clinical trial to evaluate the safety and immunogenicity of COVID-19 inactivated vaccine (Shif-Pharmed) in a healthy population. Available: https://en.irct.ir/trial/52701 [Accessed 23 Jul 2021].
19 IRCT. A double-blind, randomized, placebo-controlled phase I clinical trial to evaluate the safety and immunogenicity of COVID-19 inactivated vaccine (Shifa-Pharmed) in a healthy population aged 51-75 years. Available: https://en.irct.ir/trial/54885 [Accessed 23 Jul 2021].
20 IRCT. A double-blind, randomized, placebo-controlled phase II/III clinical trial to evaluate the safety and efficacy of COVID-19 inactivated vaccine (Shifa-Pharmed) in a population aged 18 to 75 years. Available: https://en.irct.ir/trial/54881 [Accessed 23 Jul 2021].
21 SARS-COV-2- IgM capture – Pishtazteb diagnostics. Available: http://pishtazteb.com/en/products/elisa-kits/infectious-diseases/sars-cov-2-igm-capture/ [Accessed 7 Aug 2021].
22 SARS-CoV-2 IgG – Pishtazteb diagnostics. Available: http://pishtazteb.com/en/products/elisa-kits/infectious-diseases/sars-cov-2-igg/ [Accessed 7 Aug 2021].
23 Poustchi H, Darvishian M, Mohammadi Z, *et al*. SARS-CoV-2 antibody seroprevalence in the general population and high-risk occupational groups across 18 cities in Iran: a population-based cross-sectional study. *Lancet Infect Dis* 2021;21:473–81.
24 Zhang Y, Zeng G, Pan H, *et al*. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021;21:181–92.
25 Xia S, Zhang Y, Wang Y, *et al*. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect Dis* 2021;21:39–51.
26 Ella R, Reddy S, Jogdand H, *et al*. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial. *Lancet Infect Dis* 2021;21:950–61.
27 World Health Organization. Laboratory biosafety guidance related to coronavirus disease (COVID-19): Interim guidance, 28 January 2021.
28 Alhydrogel | Croda health care. Available: https://www.crodahealthcare.com/en-gb/products-and-applications/product-finder/product/4373/Alhydrogel [Accessed 6 Aug 2021].
29 Protocol template to be used as template for observational study protocols: sentinel surveillance of adverse events of special interest (AESIs) after vaccination with COVID-19 vaccines. Available: https://apps.who.int/iris/handle/10665/342194 [Accessed 30 Dec 2021].
30 Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. Available: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical [Accessed 6 Aug 2021].
31 CTEP. Common terminology criteria for adverse events (CTCAE) | protocol development. Available: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50 [Accessed 6 Aug 2021].
32 MedDRA. English. Available: https://www.meddra.org/how-to-use/support-documentation/english [Accessed 25 Jul 2021].
33 SARS-CoV-2-Neutralizing-Ab. Available: https://pishtazteb.com/elisa-kit/sars-cov-2-neutralizing-ab/ [Accessed 24 Jul 2021].
34 SARS-COV2-RBD-IgG. Available: https://pishtazteb.com/elisa-kit/sars-cov2-rbd-igg/ [Accessed 24 Jul 2021].
35 SARS-Cov-2 Spike IgG. Available: https://pishtazteb.com/elisa-kit/sars-cov-2-spike-igg-2/ [Accessed 24 Jul 2021].
36 Antibody detection tests for COVID-19 (ELISA). Available: https://www.coronavirus-diagnostics.com/antibody-detection-tests-for-covid-19.html [Accessed 25 Jul 2021].
37 Reed LJ, Muench H. A simple method of estimating fifty per cent ENDPOINTS. *Am J Epidemiol* 1938;27:493–7.
38 Mariën J, Michiels J, Heyndrickx L, *et al*. Evaluation of a surrogate virus neutralization test for high-throughput serosurveillance of SARS-CoV-2. *J Virol Methods* 2021;297:114228.
39 Rijkers G, Murk J-L, Wintermans B, *et al*. Differences in antibody kinetics and functionality between severe and mild severe acute respiratory syndrome coronavirus 2 infections. *J Infect Dis* 2020;222:1265–9.
40 Meyer B, Reimerink J, Torriani G, *et al*. Validation and clinical evaluation of a SARS-CoV-2 surrogate virus neutralisation test (sVNT). *Emerg Microbes Infect* 2020;9:2394–403.
41 D'agostino RB, Belanger A, D'agostino RB. A suggestion for using powerful and informative tests of normality. *Am Stat* 1990;44:316–21.
42 Erjavec N. Tests for homogeneity of variance BT - international encyclopedia of statistical science. In: Lovric M, ed. Berlin, Heidelberg: Springer Berlin Heidelberg, 2011: 1595–6.
43 Cohen J. Statistical power analysis for the behavioral sciences. *Stat Power Anal Behav Sci*.
44 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* 2020;20:533–4.
45 Petrovsky N. Comparative safety of vaccine adjuvants: a summary of current evidence and future needs. *Drug Saf* 2015;38:1059–74.
46 Mulligan MJ, Lyke KE, Kitchin N, *et al*. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 2020;586:589–93.
47 Jackson LA, Anderson EJ, Rouphael NG, *et al*. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. *N Engl J Med* 2020;383:1920–31.
48 Folegatti PM, Ewer KJ, Aley PK, *et al*. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020;396:467–78.
49 Walsh EE, Frenck RW, Falsey AR, *et al*. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *N Engl J Med* 2020;383:2439–50.
50 Logunov DY, Dolzhikova IV, Zubkova OV, *et al*. Safety and immunogenicity of an RAD26 and RAD5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. *Lancet* 2020;396:887–97.
51 Ella R, Vadrevu KM, Jogdand H, *et al*. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial. *Lancet Infect Dis* 2021;21:637–46.