Efficacy and Safety of an Inactivated Virus-Particle Vaccine for SARS-CoV-2, BIV1-CovIran: Findings from a Randomised, Placebo-Controlled, Double-Blind, Multicenter Phase III Clinical Trial amid the Delta Variant Peak

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Running Title: Efficacy and safety of BIV1-CovIran in phase III clinical trial

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Summary

Objective

We report the efficacy, safety, and exploratory immunogenicity findings from a multicenter randomised placebo-controlled phase III clinical trial evaluating two 5µg doses of BIV1-CovIran vaccine.

Design

A randomised, placebo-controlled, double-blind, multicenter phase III clinical trial

Setting

In six cities of Iran, including Bushehr, Isfahan, Karaj, Mashhad, Shiraz, and Tehran. The first vaccine/placebo injection of the first participant was on May 16, 2021, in Tehran. The last vaccine/placebo injection of the last participant occurred on July 15, 2021, in Isfahan.

Participants

20,000 participants aged 18-75 years randomly assigned to the intervention/placebo groups with a ratio of 2:1.

Intervention

5µg vaccine or placebo with the interval of 28 days.

Main outcome measures

The vaccine efficacy for a 90-day follow-up period; safety, and explanatory immunogenicity assessment; variant detection during the trial.

Findings

A total of 20,000 participants were recruited in the study who were randomly assigned to receive BIV1-CovIran or placebo: 13,335 (66.7%) in the intervention and 6,665 (33.3%) in the placebo group. Participants' mean (SD) age was 38.3 (11.2) years, and 6,913 (34.6%) were female. Among vaccinated participants, a total of 758 (5.7%), 144 (1.1%) and seven (0.1%) symptomatic, severe, and critical COVID-19 cases were reported during the follow-up (median=83 days), giving an overall efficacy (95% confidence interval) of 50.2% (44.7-55.0), 70.5% (63.7-76.1) and 83.1% (61.2-93.5) against symptomatic, severe and critical cases of COVID-19, respectively. As many as two deaths were reported in the efficacy population, of which none of the death cases was among the intervention group. During the follow-up days, 41,922 adverse events were reported, of which 28,782 (68.7%) were adverse reactions; 19,363 (67.3%) in the intervention group. Most adverse reactions were mild or moderate in

severity (Grade 1 or 2) were grade 1 or 2 and self-limiting. No serious adverse events were considered to be related to the injections. For variant investigation, of 119 participants positive for SARS-CoV-2 variant, 106 (89.1%) were positive for the delta variant.

Conclusions

A two-dose regimen of BIV1-CovIran vaccine conferred 50.2%, 70.5% and 83.1% efficacy against symptomatic, severe and critical COVID-19. Vaccination was well tolerated, with no safety concerns raised.

Trial registration

Iranian Registry of Clinical Trials (IRCT20201202049567N3).

Keywords

Adverse events; Clinical trial; COVID-19 control; Double-blind method; Immunogenicity; Inactivated vaccines; Neutralising antibodies; Safety; SARS-CoV-2; Vaccination

Funding

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Introduction

The Coronavirus disease 2019 (COVID-19) pandemic has officially claimed more than 5.6 million lives globally [1]. Given the pandemic's devastating medical, economic, and social consequences, the production of effective and safe vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is considered a potential pathway for controlling the current crisis [2]. Several COVID-19 vaccines have shown promising results in phase III clinical trials so far, and vaccinations began in early 2021 [3,4]. World Health Organisation (WHO) has authorised emergency use for six vaccines and continues to evaluate additional proposals [5]. Nevertheless, the SARS-CoV-2 delta (B.1.617.2) variant, first detected in England in March 2021, rapidly became the predominant lineage due to its high transmissibility. Soon, it resulted in a higher emergency care attendance or hospital admission risk than the previous variants [6]. Moreover, there is evidence that the effectiveness of some current COVID-19 vaccines against the delta variant could be lower than the alpha variant, especially after a single vaccine dose [7].

We previously reported phase I and phase II safety and immunogenicity results from clinical trials of BIV1-CovIran vaccine [8], an inactivated whole virus particle vaccine [9]. Following the administration of the two shots of 5µg dose of BIV1-CovIran vaccine with a 28-day interval, the were no vaccine-related severe adverse events (AEs). Moreover, the vaccine significantly enhanced the immunity of all vaccine recipients against SARS-CoV-2. In phase II, the seroconversion rate of neutralising-antibody was 82.8% two weeks after the second dose [8]. These findings supported the progression of the BIV1-CovIran vaccine into phase III. Here, we report efficacy, safety, and exploratory immunogenicity findings from a multicenter randomised placebo-controlled phase III clinical trial evaluating two 5µg shots of BIV1-CovIran vaccine with a 28-day interval and a 90-day follow-up following 14 days after the second injection, with the participation of 20,000 individuals aged 18-75 years. Given that the delta variant was initially reported in Iran on June 8, 2021 [10], which overlapped with this phase III clinical trial, the results could also shed light on the vaccine efficacy on this variant.

Methods

Overview

This randomised, placebo-controlled, double-blind, multicenter phase III clinical trial was conducted to investigate the efficacy (90-day follow-up) and safety of an inactivated whole virus particle vaccine, BIV1-CovIran, among 20,000 participants aged 18-75 years located in six cities of Iran. In addition, the investigators aimed to perform an explanatory immunogenicity assessment from a sample of 400 participants in phase III. The study protocol was approved by the National Research Ethics Committee under the reference code of IR.NREC.1399.008, was registered at the Iranian Registry of Clinical Trials (IRCT20201202049567N3) and is presented in supplemental appendix 1. In this study, participants were randomly assigned to receive two intramuscular doses of BIV1-CovIran vaccine with a 28-day interval. The current vaccine's dosage selection, safety, and immunogenicity were evaluated in the phase I and phase II clinical trials [8].

Study Design

The study abided by the declaration of Helsinki, good clinical practice (GCP), and the GCP of Iran as the local regulator. All eligible volunteers were precisely thoroughly delineated about the study protocol and procedures, and written informed consent was obtained before enrollment. The data safety and monitoring board (DSMB) independently supervised the study and assisted the outcome assessors regarding the study continuation, suspension, withdrawal, and termination. All participants, outcome assessors, data managers, statisticians, and other study-related personnel were unaware of the group allocations.

The study was conducted in six cities of Iran, including Bushehr, Isfahan, Karaj, Mashhad, Shiraz, and Tehran, in predetermined vaccination centres. The first vaccine/placebo dose of the first participant was administered on May 16, 2021, in Tehran. The last vaccine/placebo injection of the last participant occurred on July 15, 2021, in Isfahan. The detailed storyline of the phase III study for each city is presented in Table 1. Figure 1 presents the mapping the timeline of phase III clinical trial with the time trend of COVID-19 weekly new cases and mortality in Iran.

Study Participants

All eligible volunteers aged 18-75 years who were capable of fully understanding the study protocol and providing written consent were invited to participate in the study using mass and social media platforms. In a pre-enrollment session, the participants were briefed about the procedures and were undergone a screening evaluation, including medical history documentation, physical examination, and laboratory

assessments. Negative real-time reverse transcription polymerase-chain-reaction (RT-PCR) was obligatory to be included in the study (supplemental appendix 1).

Sample Size

The sample size was calculated based on the WHO recommendation of achieving 150 cases across the vaccine/placebo groups, with an ultimate efficacy of 60% (lower bound of 30%). Estimating the COVID-19 incidence rate of 1% per month among the unimmunised population in Iran and 10% dropout rate, a total of 20,000 participants was required. The number of participants in each city was cardinally determined commensurate with the city population, where each trial site was located. To match the study population's age distribution to the age pyramid of the country [11], 20% of the study population in each city included participants aged 51-75 years. Participants were randomly assigned to the intervention/placebo groups within each city with a ratio of 2:1, respectively.

Randomisation and Enrollment

Using an electronic tool [12], the randomisation sheet subsuming block sequences of 3 and 6 was produced. A unique four-character randomisation code was generated upon enrollment of each eligible participant using the electronic tool [12]. Then, four other letters were added to the randomisation code: the first two letters of the participant's first name and the first two letters of their last name to form the participant's unique code. During the trial, all procedures were performed using the participant's unique code, and the identification information remained confidential by the principal investigator.

Concealment and Blinding

Vaccine and placebo vials were manufactured with the same appearance, label, and participant unique code, to ensure the blindness of participants, researchers, and outcome assessors. After the vaccine or placebo administration, the participant's unique code and administration date were written on the outer packaging box, and the label was recorded on the randomisation sheet. The study personnel checked all the information before vaccine/placebo administration. During the study, all packages were archived and maintained. In cases of any emergency events, including serious AEs, the query of emergency decoding and unblinding would urgently be requested by the principal investigator.

Intervention and Procedure

All participants in the intervention group received two shots of 0.5-millilitre doses of 5µg inactivated whole virus particle vaccine, BIV1-CovIran, manufactured by Shifa-Pharmed Industrial Group with an interval of 28 days. The SARS-CoV-2 virus used in vaccine production was isolated from the nasopharyngeal specimen of an Iranian patient with COVID-19 and had a 99.9% identity to the earliest

detected strain, Wuhan Hu-1 [13]. The vaccine was inactivated with β-propiolactone and adjuvanted with Al-hydrogel. The virus was sequenced and cultured using a Vero cell manufacturing platform in a biosafety level 3 (BSL-3) facility. Participants in the placebo group received an identical solution containing the same aluminium-hydroxide adjuvant. Vaccine and placebo vials were similar in size, shape, and colour and were stored at 2-8°C. After receiving the first dosage, individuals who experienced a temperature over 39^0C for more than three consecutive days or any severe allergic reaction and serious adverse events would not proceed to receive the second dose. In addition, participants with positive RT-PCR following the first administration were excluded from the second injection.

Assessments

Participants were monitored for 30 minutes after injection for immediate adverse events (AEs). In phase III, participants were undergone face-to-face interviews during the screening session, first injection day, and second injection day. In addition, over-the-telephone follow-up visits were held on a 14-day interval following the injection. A reactogenicity diary book was allocated to participants regarding any possible COVID-19 symptoms. All phase III participants would contact 24/7 study call centres, providing video call or file-sharing features, should they have any concerns or need medical attention using a mobile application designed for this clinical trial. Suspected COVID-19 cases were defined if participants presented (1) at least two of the following symptoms lasting for at least 48 hours: fever (axillary temperature ≥37.5°C), chills, sore throat, stuffy nose, myalgia, fatigue, headache, nausea or vomiting, or diarrhoea or (2) at least one respiratory sign or symptom (including cough, shortness of breath), new olfactory or taste disorder, or radiographic evidence of COVID-19–like pneumonia. Upon the report of any suspicious COVID-19 symptoms, a nasopharyngeal specimen would be obtained at the clinical trial site, and RT-PCR would be performed at a central laboratory. In cases of negative RT-PCR tests, participants underwent further RT-PCR tests after 48 hours unless their symptoms regressed. Positive RT-PCR tests would indicate definitive symptomatic COVID-19. COVID-19 severity status was categorised as symptomatic, severe, and critical, based on the diagnosis scheme from the WHO [14] (supplemental appendix 1).

Study Endpoints

Efficacy Endpoints

The efficacy of BIV1-CovIran was primarily assessed by the onset of definitive symptomatic COVID-19 infection during a 90-day follow-up following 14 days after the second injection. Participants received two doses of the vaccine candidate on days zero and 28. The follow-up of participants began 14 days

after the second dose (day 42) to day 132 (90-day follow-up period). The onset of severe and critical COVID-19 among participants of phase III and deaths due to COVID-19 during the 90-day follow-up was assessed as the secondary efficacy endpoints. In addition, we evaluated the post hoc efficacy of the BIV1-CovIran vaccine for COVID-19 symptomatic cases, hospitalisation and death by subgroups, including sex, age, serology, and location.

Safety Endpoints

Immediate adverse events (AEs) were assessed at the injection site by monitoring participants 30 minutes after injection in the trial centre. All participants were required to report all local and systematic adverse reactions (ARs) and AEs following the injection using the trial's mobile application. Solicited ARs were defined as any events which occurred from day zero to day seven after each injection. Unsolicited ARs were defined as any ARs which occurred from day eight to day 28 after each injection. The severity of ARs was defined using the Food and Drug Administration (FDA) Guidance for Industry and Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials [15].

Exploratory Immunogenicity Endpoints

To further evaluate the immunogenicity enhancement of the BIV1-CovIran vaccine among phase III participants, the humeral response against SARS-CoV-2 in a subsample of 400 participants in Tehran was assessed using the convenience sampling method. The first 400 participants of Tehran location were chosen to be assessed for exploratory immunogenicity response. The participants provided a separate informed consent for this procedure. We evaluate the geometric mean titers (GMT), geometric mean ratios (GMR) of antibodies against SARS-CoV-2 and the seroconversion rates 14 days after the second injection. The blood samples were collected from the participants before the first injection and on day 14 after the second injection. Neutralising, anti-receptor binding domain (RBD), and anti-spike glycoprotein antibodies were measured using Enzyme-linked Immunosorbent Assay (ELISA) kits [8]. Seroconversion was defined as a post-vaccination IgG titer that was at least four-fold higher than the baseline titer.

Dominant Variant

Between August 8th 2021 to October 13th 2021, a sub-sample of symptomatic phase III participants from Tehran trial site were selected via convenience sampling method for investigating the dominant variant during the trial.

Statistical Analysis

Vaccine efficacy was calculated using a Poisson regression compared to the placebo group, defining the dependent variable as the number of incident cases, the independent variable as the treatment group, and the offset as the person-years. Efficacy analysis was performed on the efficacy population, who were fully vaccinated and had a vaccine/placebo administration interval of 28 ±3 days. Efficacy was assessed by the onset of COVID-19 among the efficacy population who were followed for 90 days following 14 days after the second dose. The maximum contribution of each participant to person-time equalled 0.246 (90/365) person-years. Simultaneously, the vaccine efficacy against severe and critical cases of COVID-19 and deaths due to COVID-19 was analysed. Considering the study population size during this phase III clinical trial, it was assumed that missing covariates at baseline would not significantly affect the vaccine efficacy calculation and were not imputed.

$$Vaccine\ efficacy\ (\%) = \left(1 - \frac{\text{Incidence density of intervention group}}{Incidence\ density\ of\ placebo\ group}\right) \times 100$$

$$Incidence\ density = \left(\frac{\#\ confirmed\ cases\ during\ the\ effective\ follow-up}{\#observation\ years\ of\ all\ vaccinated\ participants}\right) \times 100$$

In addition, an exploratory post hoc analysis was performed to assess the subclass group vaccine efficacy analysis based on study sites, sex, age groups, and baseline serology (IgG or IgM) status. Interaction testing between subgroups was not performed due to limited statistical power.

Safety analysis was performed among the safety population, defined as participants who received at least one injection dose throughout the study. The exploratory humoral immunogenicity assessment was conducted for a subgroup of participants who had randomly received the vaccine/placebo with blood collection before and after each injection. Frequency (per cent), mean, and standard deviation (SD) were used to elucidate the data. The groups were compared with a two-sample t-test at a two-sided 5% significance level. The statistical analyses were carried out using R statistical packages v3.4.3 (http://www.r-project.org, RRID: SCR_001905). Data visualisations were performed using Tableau Desktop, version 2020.1, an interactive data visualisation software.

Funding Sources

The study was supported by the Shifa-Pharmed Industrial Group (grant number 63971). The sponsor was blinded and had no role in study design, data collection, analysis, interpretation, manuscript drafting, or submission. An academic contract research organisation (CRO) affiliated with the Clinical Trial Center,

Tehran University of Medical Sciences, Tehran, Iran, was in charge of clinical trial management and monitoring. The unmasked randomisation list was not shared with the study sponsor. An independent third-party research centre (Non-Communicable Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran) performed the data cleaning and analysis and drafted the manuscript.

Patient involvement

The public was not involved in setting the research question, the outcome measures, the design or implementation of the study. Outcomes will be disseminated through study newsletters, community events, social media, and media releases.

Results

Characteristics of participants

As many as 64,049 volunteers in all study sites were screened for eligibility, and 20,000 were randomised: 13,335 (66.7%) in the intervention and 6,665 (33.3%) in the placebo group (Figure 2). All included participants received the first dose, among whom 19,506 (97.5%) received the second dose (13,011 (97.6%) in the intervention group and 6,495 (97.4%) in the placebo group). Of all participants, 6,458 (32.3%) were seropositive for baseline IgG or IgM tests; 4,305 (32.3%) in the intervention and 2,153 (32.3%) in the placebo group, which were excluded in the subclass analysis of vaccine efficacy. As many as 105 (0.5%) of fully vaccinated participants were excluded due to deviation from study protocol, and the data of 19,401 (99.5%): 12,945 in the intervention group and 6,456 in the placebo group were analysed for vaccine efficacy (Figure 2). All 20,000 participants who received at least one dose of vaccine or placebo were included in the safety population.

Baseline characteristics of phase III participants are presented in Table 2. Participants' mean (SD) age was 38.3 (11.2) years, and 6,913 (34.6%) were female. As many as 1,428 (7.1%) of recruited participants had at least one coexisting underlying condition, with 932 (7.0%) in the intervention and 496 (7.4%) in the placebo group. Considering the approximation of the study demographics with the population structure in Iran, nearly 17% of participants in both intervention and placebo groups were aged more than 50 years. A total of 8,716 (43.6%) participants included in the study were from Tehran, 2,867 (14.3%) from Mashhad, 2,671 (13.4%) from Isfahan, 2,328 (11.6%) from Shiraz, 1,985 (9.9%) from Karaj, and 1,433 (7.2%) from Bushehr.

Vaccine Efficacy

Primary Efficacy Endpoint Analysis

Of 19,404 participants in the efficacy population, as many as 1,446 (7.5%) symptomatic COVID-19 cases were confirmed during the 90-day follow-up following 14 days after the second dose (median follow-up duration of 83 days), with 758 (5.9%) in the intervention group and 688 (10.6%) in the placebo group. The incidence rate of COVID-19 was 242.4 (95% confidence interval (CI), 225.4-260.3) per 1000 person-years was in the intervention group and 486.1 (95% CI, 450.5-523.8) per 1000 person-years in the placebo group. This case split corresponds to 50.2% (95% CI, 44.7-55.0) vaccine efficacy. Of participants with COVID-19 infection, 365 (1.9%) participants met the criteria of severe COVID-19, with 144 (1.1%) in the intervention group. This case split corresponds to 70.5% (95% CI, 63.7-76.1) vaccine efficacy. Among participants, seven cases of critical COVID-19 with onset 90-day follow-up

following 14 days after the second dose were observed among vaccine recipients and 19 among placebo recipients. This case split corresponds to 83.1% (95% CI, 61.2-93.5) vaccine efficacy. There were no death cases due to COVID-19 with onset 90-day follow-up among vaccine recipients, while there were two cases of mortality due to COVID-19 among the placebo recipients. Considering the small number of incident death cases, these results must be interpreted with caution (Table 3).

Post Hoc Analysis

A similar number of COVID-19 symptomatic cases were observed for men and women (Table 4). Of all critical cases, five were female, one in the intervention group and four in the placebo group. Among males with critical COVID-19, six were in the intervention and 15 placebo groups. Considering the limited statistical power, the interpretation of these findings was not possible.

Among participants aged 51-75 years, there were 221 with positive COVID-19 RT-PCR test consisting of 105 among vaccinated participants. Only two participants aged 51-75 years were included in the critical COVID-19 cases.

Among participants aged 65-75 years, 46 (16.7%) symptomatic COVID-19 cases were recorded, with only 8 (17.4%) in the intervention group. The vaccine efficacy among participants aged 65-75 years for symptomatic COVID-19 was 90.7 (95% CI, 81.0-96.0). There were 18 severe and only one critical COVID-19 cases among participants aged 65-75 years cases. Interpretation of these subgroup findings is limited in the absence of tests for interaction due to limited statistical power.

The number of symptomatic COVID-19 events for each location is presented in Table 4. No death cases of COVID-19 occurred among vaccinated participants in either group of sex, age, and location; thus, efficacy was not calculated. Given the serology status of phase III participants, the vaccine efficacy among seronegative participants against symptomatic, severe, and critical COVID-19 infection, was 42.9 (95% CI, 35.8-49.1), 63.7 (95% CI, 54.0-71.4), and 82.8 (95% CI, 54.7-94.6), respectively (Table S1).

Vaccine Safety

Local and systemic AEs in the intervention and placebo groups are presented in Table 5. During the follow-up days, 41,922 AEs were reported, of which 28,782 (68.7%) were ARs; and 19,363 (67.3%) were in the intervention group. As many as 17,626 (61.2%) ARs were solicited consisting of 11,850 (61.2%) in the intervention group and 5,777 (61.3%) in the placebo group.

Of all 20,000 participants, ARs occurred among 11,776 (58.9%) participants, with 7,960 (59.6%) in the intervention group and 3,816 (57.4%) in the placebo group. The most common local AR among phase III participants was pain in the injection site, which accounted for 38.0% of all total ARs in the

intervention group and 35.5% in the placebo group. Headache with 12.3% of total ARs in the intervention group and 12.4% in the placebo group, was considered the most common systemic AR among phase III participants. Most ARs were mild or moderate in severity (Grade 1 or 2) and were transient and self-limiting, without the need for special consideration (Table 5 and Table S2). The mean (SD) number of days to resolve the ARs were 2.7 (4.9).

A total of 164 serious AEs (all causes) occurred during follow-up, with 85 (51.8%) occurrences in the intervention group (Table S3). No serious AEs were considered to be related to the injections. Throughout the three-month follow-up of phase III participants, five deaths due to COVID-19 infection were reported, of which two were in the efficacy population (Table S4).

Exploratory immunogenicity analysis

Among 400 vaccinated participants who were selected for exploratory immunogenicity analysis, the GMTs of the neutralising, anti-RBD and anti-S glycoprotein antibodies at baseline in the intervention group were 1.4 (1.3, 1.6), 0.5 (0.4, 0.6) and 1.2 (0.9, 1.6), respectively; while the corresponding rates were 6.0 (5.0, 7.1), 8.3 (7.5, 9.2) and 49.4 (43.7, 55.9) on day 14 after the second dose. In the placebo group, the GMTs for neutralising, anti-RBD and anti-S glycoprotein antibodies at the baseline were 1.4 (1.1, 1.6), 0.6 (0.4, 0.8), and 1.4 (0.9, 2.2), respectively. On day 42, the corresponding GMTs were 0.9 (0.7, 1.1), 0.6 (0.4, 0.8), and 1.3 (0.8, 2.0), respectively. The seroconversion rate for neutralising antibodies was 64.4% (58.3, 70.2) in the intervention group versus 8.1% (4.1, 14.0) in the placebo group (Table 6, Figure 3). The exploratory immunogenicity analysis results among seronegative participants are presented in Table S5.

Dominant variant

Among 151 samples, 119 (78.8%) samples were positive for SARS-CoV-2, and 32 (21.2%) were negative. When checked for the SARS-CoV-2 variant, 106 samples (89.1%) were positive for the Delta variant; 6 (5.0%) for the Wuhan variant; 6 (5.0%) for the Alpha variant, and 1 (0.9%) for Gamma, Lambda, or Beta variant.

Discussion

This study presents the findings from the phase III clinical trial of BIV1-CovIran, an inactivated whole virus particle vaccine for SARS-CoV-2, adjuvanted with aluminium hydroxide. Vaccine efficacy was assessed during a 90-day follow-up following 14 days after the second dose. Based on the final per-protocol analysis, a two-dose regimen of the vaccine (5µg per dose, given 28 days apart) was well-tolerated, induced significant seroconversion, was 50% effective against symptomatic COVID-19, and was 83% effective against critical COVID-19. Moreover, there were no deaths in the vaccine group during the follow-up period. Our preliminary vaccine efficacy against symptomatic COVID-19 infection was 50%, with a vaccine efficacy of 91% among participants aged 65-75 years. Thus, the vaccine efficacy greatly exceeded the primary efficacy endpoints as set by WHO [16].

The favourable safety profile of BIV1-CovIran observed in the phase I and phase II clinical trials [8] was confirmed in phase III. In this study, no safety concerns were raised, no anaphylactic events after BIV1-CovIran administration were reported, and all AEs (solicited, unsolicited, and serious adverse events) were well balanced between the intervention and placebo groups. The most common AE was injection site pain, followed by headache. As many as 164 serious AEs were reported during the trial, of which 85 was in the intervention group, and all were non-vaccine related AEs. Serious AEs in this study were defined as death, life-threatening events, hospitalisation due to any cause, prolonged hospitalisation period, and any other conditions deemed serious by the principal investigator. As presented in Table S3, 100 of 164 serious AEs were hospitalisations due to COVID-19: 53 in the placebo group and 47 in the intervention group. a potential explanation for higher rates of serious AEs in the placebo group could be the partial protection against COVID-19 hospitalisation among participants who received the vaccine. The combined incidence of local and systemic AEs after both vaccine doses in this study was similar to that of other inactivated SARS-CoV-2 vaccines [17–21], and lower than that of other SARS-CoV-2 vaccine platforms [22–27]. Nevertheless, such a comparison of AEs among vaccines of different platforms needs to be interpreted with caution, and further studies are required for a head-to-head comparison of the short-term and long-term safety across all SARS-CoV-2 vaccine platforms.

This phase III study was done during a period that included the fifth wave of COVID-19 infections in Iran, mainly consisting of delta variant of SARS-CoV-2, with a peak of more than 50,000 new cases per day [10,28]. The emergence of the delta variant rapidly resulted in a significant rise of COVID-19 cases worldwide [28] as well as hospital admissions, far beyond what was already witnessed with the alpha variant. There is evidence that the substantial mutations [29] have resulted in increased transmission [30] of the delta variant. Moreover, some mutations could inversely affect the host immune responses [31].

While the unvaccinated populations were the most vulnerable to the delta variant [6], it also challenged the effectiveness of COVID-19 vaccines, especially after receiving a single dose [32]. When a sub-sample of participants with symptomatic COVID-19 were assessed for the delta variant, 89.1% were positive for the delta variant, suggesting that the dominant variant was the delta variant during the study. In our preliminary analysis, we found a vaccine efficacy of 50% against symptomatic COVID-19, and 83% against critical COVID-19 at the time of study. Assuming that the dominant variant was delta during the study, BIV1-CovIran could have had an acceptable efficacy against this variant. Nevertheless, further investigations are necessary to confirm clinical efficacy against this and other potential SARS-CoV-2 variants in the future.

Initially reported in late 2021, the highly contagious B.1.1.529 (Omicron) variant of concern has globally outcompeted the earlier variants with its higher rates of spike protein mutation, resulting in higher immune evasion capacity [33,34]. The Omicron variant has reportedly challenged the effectiveness and neutralisation capacity of SARS-CoV-2 vaccines, developed initially against the Wuhan variant [33,35–38]. Among the potential mitigating strategies for Omicron or future variants of concern, choosing between delivering booster doses of conventional COVID-19 vaccines, homologous or heterologous [39], and developing Omicron-based vaccine boosters [40–42] is an ongoing debate which needs extensive investigation in the future studies.

Our efficacy and safety findings were comparable with previous results of two other alum-adjuvanted inactivated SARS-CoV-2 vaccines, which both showed 78% [21,43]. Thus, full vaccination with BIV1-CovIran could potentially result in a marked reduction of cases with critical COVID-19 and death due to COVID-19, which put a tremendous burden on healthcare systems and take a heavy toll on populations.

Strengths and limitations

Based on the follow-up data, BIV1-CovIran was safe and effective against SARS-CoV-2 infection, preventing severe and critical cases. Considering the catastrophic toll of COVID-19 in Iran and the emerging evidence of the need for booster doses [44], the public rollout of a safe domestic COVID-19 vaccine could be a valuable solution. We also acknowledge the limitations of the study. This study presents the results of a three-month follow-up after the second dose of BIV1-CovIran. Thus, the occurrence of AEs beyond the study period and evidence on efficacy and the duration of protection remain to be determined in future studies. Since vaccine efficacy tends to wane over time [45], the efficacy estimates would likely drop if the trial had a longer follow-up time. Nevertheless, this needs further investigation in future studies. The phase III trial was designed to follow participants for safety and efficacy for 365 days after the second dose (supplemental appendix 1). However, ethical

considerations prevented following placebo recipients for 365 days without active immunisation. Once approved vaccines for COVID-19 were publically available, the national ethical committee required all clinical trials for COVID-19 vaccines to decode their data and notify participants in the placebo group to get vaccinated for COVID-19, after three months of the second placebo administration. Subsequently, participants in the placebo group were administered the currently available COVID-19 vaccines. We initially aimed to present the interim analysis based on the 180-days follow-up, as stated in the study protocol. After the request of the ethical committee, we provided the interim report for the Iranian Food and Drug Administration based on a shorter follow-up period (median= 83 days) after the second dose, the results of which are presented in the current study. The follow-up duration of other COVID-19 vaccine phase III trials after the second dose was 99 days (median) for BBV152 [21], 112 days (median) for BBIBP-CorV [46], 61 days (median) for ChAdOx1 nCoV-19 [47], 2 months (median) for ChAdOx1 nCoV-19 [26], six months for BNT162b2 [48]. Moreover, this was 90 days after the first dose for CoronaVac [49]. The study did not have enough power to assess efficacy by subgroup definitively. Despite being a multicenter trial, all the clinical trial phases of BIV1-CovIran have been conducted in Iran. We reported the immunogenicity of the vaccine candidate in phase I/II studies [8]. In the phase III trial, we assessed the exploratory immunogenicity response of 400 participants using convenience sampling method. Therefore, the results might not be representative of the general population, which needs to be assed in future studies. The vaccine has not been studied on ethnicities beyond the Iranian population, which needs to be addressed in future studies. The study population also lacked ethnic and racial diversity, highlighting the importance of evaluating the efficacy of BIV1-CovIran in other populations. This report does not address the prevention of COVID-19 in different populations, such as younger adolescents, children, and pregnant women. Given the occurrence of the SARS-CoV-2 Delta variant amid this phase III trial [10], variant detection was conducted in the study. Due to resource constraints in other trial sites, this procedure was only available in Tehran, the main trial site with 43.6% of participants. Using the convenience sampling method, 151 samples were obtained from 913 participants at the Tehran trial site who became symptomatic of COVID-19 and underwent variant detection.

Conclusion

BIV1-CovIran had high efficacy against severe and critical COVID-19. Vaccination was well tolerated, with no safety concerns raised.

What is already known on this topic

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 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. The phase I and phase II clinical trials of the BIV1-CovIran showed a potentially safe and immunogenic vaccine candidate. The efficacy of this vaccine against symptomatic, severe, critical COVID-19 disease, and deaths due to COVID-19 infection, has not been previously investigated.

What this study adds

The present study reports preliminary results of randomised, placebo-controlled, double-blind, multicenter phase III clinical trial of BIV1-CovIran vaccine among 20,000 participants in six cities of Iran to assess the safety and efficacy of the vaccine against symptomatic, severe, critical, and death cases due to COVID-19 amid delta variant surge. In this study, all participants received two shots of BIV1-CovIran at a 28-day interval. Vaccine efficacy, assessed during a 90-day follow-up, against severe and critical COVID-19 infection was 70.5% (95% CI, 63.7-76.1) and 83.1% (61.2-93.5), respectively, with a 100% protection against mortality in vaccinated participants. Our preliminary vaccine efficacy against symptomatic COVID-19 infection was 50.2% (44.7-55.0); with a vaccine efficacy of 90.7% (81.0-96.0) among participants aged 65-75 years.

Ethical statement

Ethical approval

The study protocol was approved by the National Research Ethics Committee under the reference code of IR.NREC.1399.008, and was registered at the Iranian Registry of Clinical Trials (IRCT20201202049567N3).

Data availability statement

De-identified, individual participant data will be made available when the trial is complete, upon 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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Footnotes

Contributors

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Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: As the principal investigator, Minoo Mohraz received funding for project administration from Shifa-Pharmed Industrial Group (grant number 63971). Asghar Abdoli is the founder and the scientific director of Amirabad Virology Lab and the only shareholder of this laboratory. He is a faculty member of the Pasteur Institute of Iran and was also a project consultant for the PastoCoAd vaccine

project, which was initiated after the BIV1-CovIran vaccine project. Payam Tabarsi was the principal investigator of another SARS-CoV-2 vaccine trial, SpikoGen. As the principal investigator. Hamed Hosseini was the manager of the Clinical Trial Center (CTC), an academic CRO affiliated to Tehran University of Medical Sciences, and he was responsible for the conduct and monitoring of clinical trials. He was also a non-voting member of DSMB, which was mandated by national regulatory. All other co-authors declare no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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Abbreviations

AEs = Adverse Events

AR = Adverse Reaction

BSL-3 = Biosafety Level 3

CI = Confidence Interval

COVID-19 = Coronavirus Disease-2019

CRO = Contract Research Organisation

ELISA = Enzyme-linked Immunosorbent Assay

FDA = Food and Drug Administration

GCP = Good Clinical Practice

GMR = Geometric Mean Ratio

GMT = Geometric Mean Titer

IgG = Immunoglobulin G

IgM = Immunoglobulin M

MedDRA = Medical Dictionary for Regulatory Activities

RBD = Receptor Binding Domain

RT-PCR = Reverse Transcription Polymerase Chain Reaction

SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2

SD = Standard Deviation

VE = Vaccine Efficacy

WHO = World Health Organisation

Tables

Table 1. Timeline of phase III clinical trial in each city

City	First injection		Second injection	
	Start day	End day	Start day	End day
Tehran	16 May 2021	31 May 2021	13 June 2021	28 June 2021
Mashhad	10 June 2021	17 June 2021	08 July 2021	15 July 2021
Isfahan	10 June 2021	17 June 2021	08 July 2021	15 July 2021
Shiraz	09 June 2021	16 June 2021	07 July 2021	14 July 2021
Karaj	26 May 2021	05 June 2021	23 June 2021	03 July 2021
Bushehr	09 June 2021	16 June 2021	07 July 2021	15 July 2021

Table 2. Baseline characteristics of participants in phase III clinical trial of BIV1-CovIran vaccine among 18-75-year participants

Study population	Intervention (n= 13,335)	Placebo (n= 6,665)
Age (years), mean (SD)	38.3 (11.2)	38.2 (11.1)
Age groups, n (%)		
18-50 years	11,035 (82.8)	5,564 (83.5)
51-75 years	2,300 (17.2)	1,101 (16.5)
Sex, n (%)		
Female	4,606 (34.5)	2,307 (34.6)
Male	8,729 (65.5)	4,358 (65.4)
Study site, n (%)		
Tehran	5,812 (43.6)	2,904 (43.6)
Mashhad	1,911 (14.3)	956 (14.3)
Isfahan	1,781 (13.4)	890 (13.4)
Shiraz	1,552 (11.6)	776 (11.6)
Karaj	1,324 (9.9)	661 (9.9)
Bushehr	955 (7.2)	478 (7.2)
Height (cm), mean (SD)	172.0 (9.5)	171.9 (9.5)
Weight (kg), mean (SD)	78.9 (16.1)	78.6 (16.1)
BMI, mean (SD)	26.5 (4.5)	26.5 (4.5)
Comorbidity, n (%)		
Cardiovascular disease	728 (5.5)	367 (5.5)
Diabetes and other endocrinology disease	310 (2.3)	173 (2.6)
Liver and gastrointestinal	27 (0.2)	17 (0.3)
Respiratory disease	13 (0.1)	6 (0.1)
Other	99 (0.7)	28 (0.4)
None	12,403 (93.0)	6,169 (92.6)
Positive baseline IgG antibody, n (%)	2825 (21.2)	1488 (22.3)
Positive baseline IgM antibody, n (%)	1480 (11.1)	665 (10.0)

Table 3. Efficacy of BIV1-CovIran vaccine for various COVID-19 outcomes

Outcome	Intervention	Placebo
Number of participants	12,945	6,456
Person-years	3,126.8	1,414.8
Symptomatic COVID-19 infection		
Number of incident cases	758	688
Incidence density per 1000 person-years, (95% CI)	242.4 (225.5-260.3)	486.3 (450.6-524.0)
Vaccine efficacy, % (95% CI)	50.2 (44.7-55.0)	N/A
Severe COVID-19 infection		
Number of incident cases	144	221
Incidence density per 1000 person-years, (95% CI)	46.1 (38.8-54.2)	156.2 (136.3-178.2)
Vaccine efficacy, % (95% CI)	70.5 (63.7-76.1)	N/A
Critical COVID-19 infection		
Number of incident cases	7	19
Incidence density per 1000 person-years, (95% CI)	2.2 (0.9-4.6)	13.4 (8.1-21.0)
Vaccine efficacy, % (95% CI)	83.1 (61.2-93.5)	N/A
Death due to COVID-19		
Number of incident cases	0	2
Incidence density per 1000 person-years (95% CI)	0	1.4 (0.2-5.1)
Vaccine efficacy, % (95% CI)	N/A	N/A

Table 4. Symptomatic COVID-19 cases among study subgroups in the intervention and the placebo groups

Subgroups	Intervention group n (%)	Placebo group n (%)
Sex		
Female	253 (5.7)	216 (9.7)
Male	505 (6.0)	253 (6.0)
Age groups		
18-50 years	653 (6.1)	572 (10.6)
51-75 years	105 (4.7)	116 (10.9)
Location		
Tehran	498 (8.9)	415 (15.8)
Mashhad	55 (3.0)	51 (5.5)
Isfahan	28 (1.6)	76 (8.8)
Shiraz	41 (2.7)	48 (6.3)
Karaj	97 (7.6)	74 (11.6)
Bushehr	39 (4.2)	24 (5.1)
Serology status		
Seronegative	632 (6.7)	520 (10.8)
Seropositive	126 (3.6)	168 (10.3)
Comorbidity		
Yes	53 (5.9)	52 (10.9)
No	705 (5.9)	636 (10.6)

Table 5. Adverse reactions (ARs) after administration of BIV1-CovIran/placebo in the safety population

Adverse events (AEs)	Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
	Events	Participants n (%)	Events	Participants n (%)	Events	Participants n (%)
Adverse Reactions						
Total	19,363	7,960 (59.7)	9,419	3,816 (57.3)	28,782	11,776 (58.9)
Solicited	11,850	6,129 (46.0)	5,777	2,932 (44.0)	17,626	9,061 (45.3)
Unsolicited	7,517	4,277 (32.1)	3,639	2,099 (31.5)	11,156	6,376 (31.9)
Systemic	12,866	5,272 (39.5)	6,402	2,512 (37.7)	19,268	7,784 (38.9)
Local	6,497	5,189 (38.9)	3,017	2,428 (36.4)	9,514	7,617 (38.1)
Grading of Adverse Reactions						
Grade 1	18,131	7,820 (58.6)	8,850	3,773 (56.6)	26,981	11,593 (58.0)
Grade 2	1,126	743 (5.6)	528	330 (5.0)	1654	1,073 (5.4)
Grade 3	104	75 (0.6)	43	32 (0.5)	147	107 (0.5)
Grade 4	0 (Zero)	0 (Zero)	0 (Zero)	0 (Zero)	0 (Zero)	0 (Zero)

Table 6. Exploratory immunogenicity assessment two weeks after the second dose among 400 participants of phase III

Antibody	Geometric mean titer (µg/ml) (95% CI)		Geometric mean ratio (95% CI)	Seroconversion rate* (%) (95% CI)	
	5µg	Placebo	5µg	5µg	Placebo
Neutralising antibody					
Day 0	1.4 (1.3, 1.6)	1.4 (1.1, 1.6)	1.1 (0.9, 1.3)	–	–
Day 42	6.0 (5.0, 7.1)	0.9 (0.7, 1.1)	6.6 (5.0, 8.8)	64.4 (58.3, 70.2)	8.1 (4.1, 14.0)
Difference	10.76 (8.77, 12.75)	-0.09 (-1.47, 1.28)	N/A	N/A	N/A
Anti-receptor binding domain IgG					
Day 0	0.5 (0.4, 0.6)	0.6 (0.4, 0.8)	1.3 (0.9, 1.8)	–	–
Day 42	8.3 (7.5, 9.2)	0.6 (0.4, 0.8)	15.0 (11.6, 19.6)	66.3 (60.2, 72.0)	14.7 (9.2, 21.8)
Difference	7.55 (6.9, 8.19)	0.44 (-0.4, 1.28)	N/A	N/A	N/A
Anti-spike glycoprotein IgG					
Day 0	1.2 (0.9, 1.6)	1.4 (0.9, 2.2)	0.9 (0.5, 1.5)	–	–
Day 42	49.4 (43.7, 55.9)	1.3 (0.8, 2.0)	38.4 (26.8, 54.9)	71.2 (65.3, 76.6)	14.0 (8.6, 21.0)
Difference	50.16 (45.05, 55.27)	0.74 (-5.82, 4.34)	N/A	N/A	N/A

Results reported at baseline (day 0) and two weeks after the second vaccination (day 42) for 5µg and placebo groups.
*Defined as a post-vaccination IgG titer that was at least four-fold higher than the baseline titer. Geometric mean titers for neutralising antibodies are reported in µg/ml-anti-receptor binding domain IgG in RU/ml-and anti-spike glycoprotein IgG RU/ml.
**Not applicable

Figure legends

Figure 1. Mapping the timeline of phase III clinical trial with the time trend of COVID-19 weekly new cases (blue line) and mortality (red line) in Iran by mid-May 2022.

Figure 2. Diagram of screening, enrollment, randomisation, and follow-up in phase III.

Figure 3. Anti-SARS-CoV-2 antibody titres for neutralising (a), anti-RBD (b), and anti-spike (c) antibodies in phase III. Data points and quartiles are presented in box plots.

Print abstract

Study question

To assess the efficacy and safety of two doses of BIV1-CovIran SARS-CoV-2 vaccine in a multicenter randomised placebo-controlled phase III clinical trial.

Methods

This randomised, placebo-controlled, double-blind, multicenter phase III clinical trial aimed to investigate the efficacy (90-day follow-up) and safety of an inactivated whole virus particle vaccine, BIV1-CovIran, among 20,000 participants aged 18-75 years located in six cities of Iran. Participants were randomly assigned to receive two intramuscular doses of BIV1-CovIran vaccine/placebo with a 28-day interval.

Study answer and limitations

Among vaccinated participants, a total of 758 (5.7%), 144 (1.1%) and seven (0.1%) symptomatic, severe, and critical COVID-19 cases were reported during the follow-up (median=83 days), giving an overall efficacy (95% confidence interval) of 50.2% (44.7-55.0), 70.5% (63.7-76.1) and 83.1% (61.2-93.5) against symptomatic, severe and critical cases of COVID-19, respectively. During the follow-up days, 41,922 adverse events were reported, of which 28,782 (68.7%) were adverse reactions; 19,363 (67.3%) in the intervention group. Most adverse reactions were mild or moderate in severity (Grade 1 or 2) were grade 1 or 2 and self-limiting. No serious adverse events were considered to be related to the injections. For variant investigation, of 119 participants positive for SARS-CoV-2 variant, 106 (89.1%) were positive for the delta variant. The occurrence of adverse events beyond the study period and evidence on efficacy and the duration of protection remain to be determined in future studies.

What this study adds

Our preliminary vaccine efficacy against symptomatic COVID-19 infection was 50.2% (44.7-55.0); with a vaccine efficacy of 90.7% (81.0-96.0) among participants aged 65-75 years.

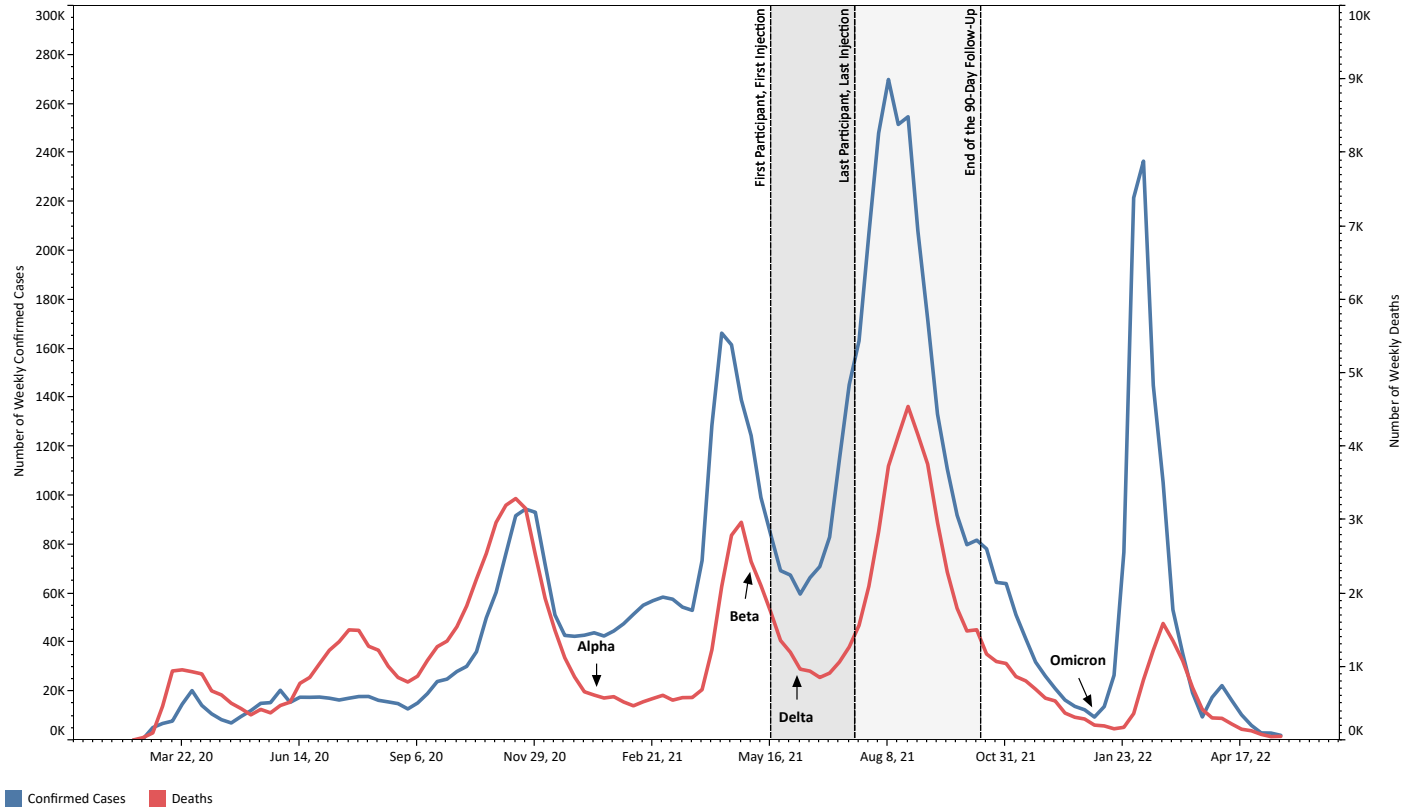
Funding, competing interests, data sharing

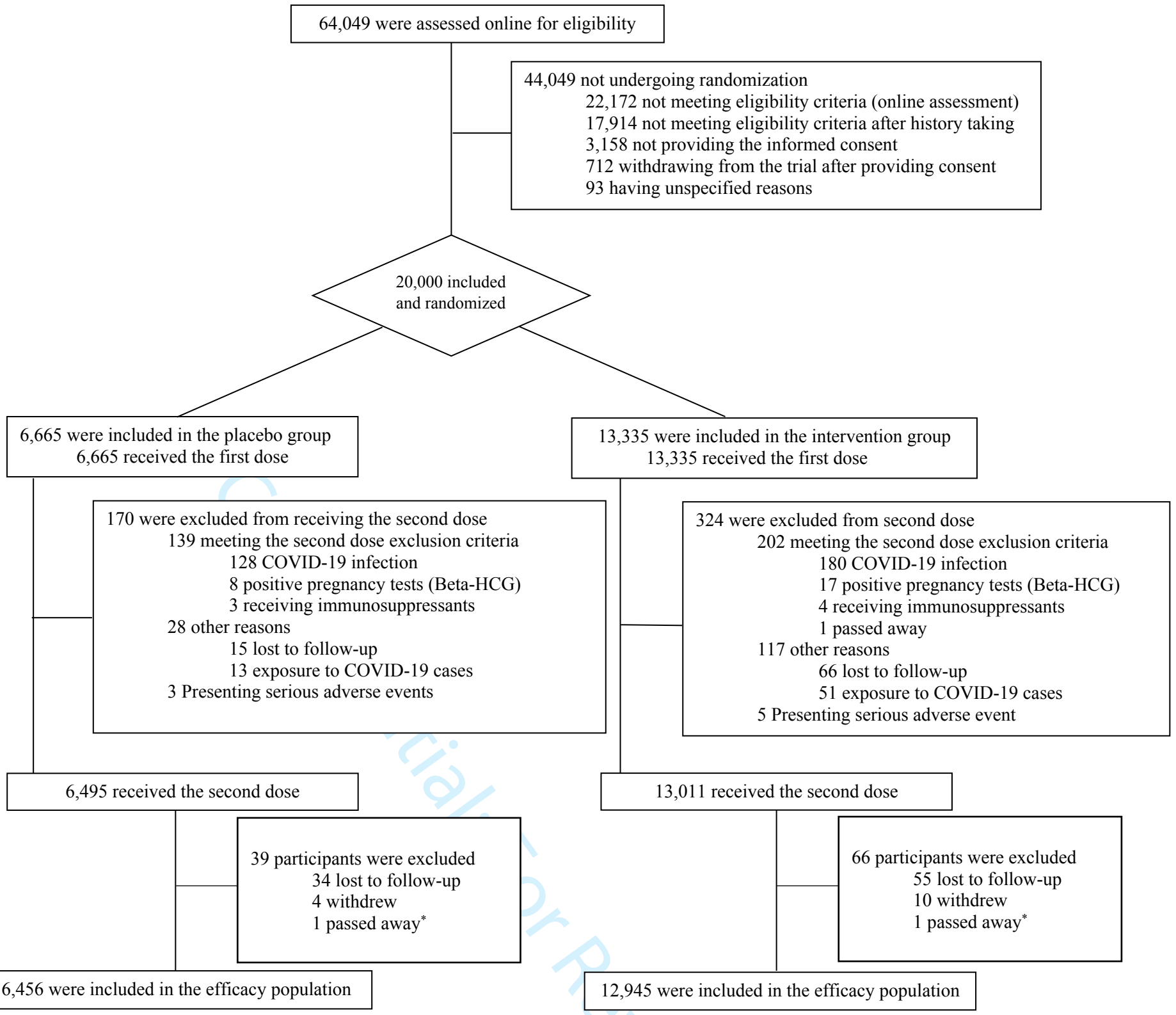
Shifa-Pharmed Industrial Group, grant number 63971; Minoo Mohraz: received funding for project administration from Shifa-Pharmed Industrial Group. Asghar Abdoli: the founder and the scientific director of Amirabad Virology Lab and the only shareholder of this laboratory. A faculty member of the Pasteur Institute of Iran and a project consultant of another SARS-CoV-2 vaccine trial. Payam Tabarsi:

principal investigator of another SARS-CoV-2 vaccine trial. Hamed Hosseini: Clinical Trial Center manager, and a non-voting member of DSMB. All other co-authors: none declared.

Study registration

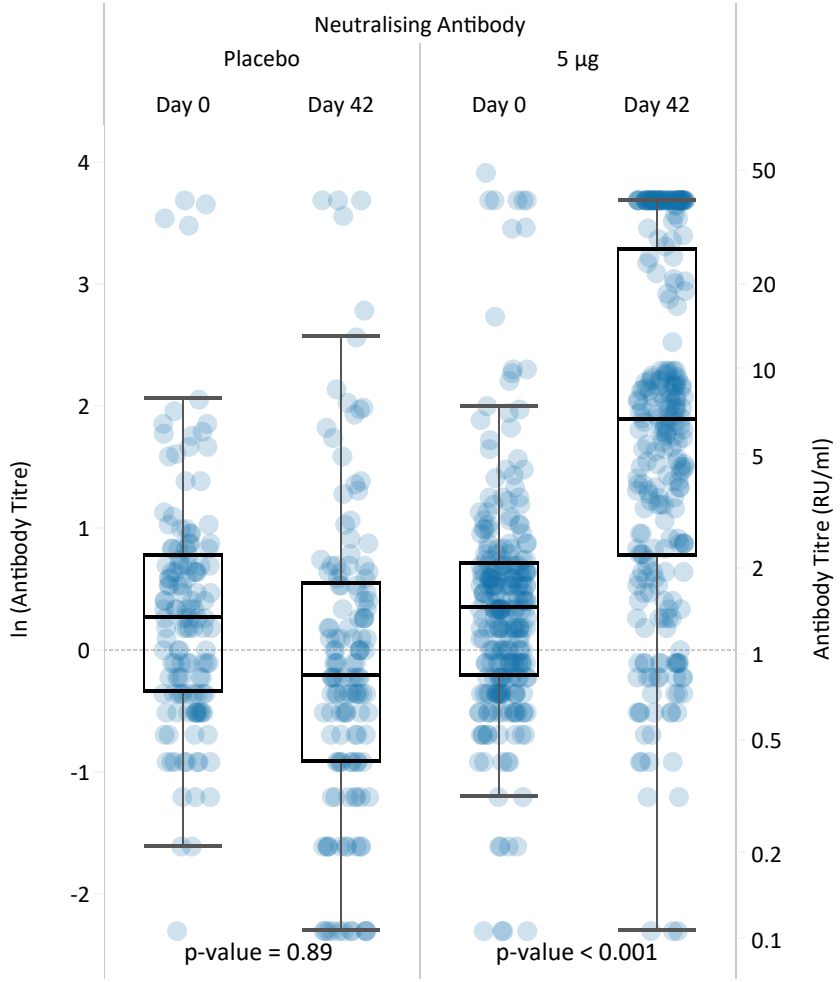
Iranian Registry of Clinical Trials (IRCT20201202049567N3).


Number of Weekly Confirmed Cases
Number of Weekly Deaths
300K
280K
260K
240K
220K
200K
180K
160K
140K
120K
100K
80K
60K
40K
20K
0K
10K
9K
8K
7K
6K
5K
4K
3K
2K
1K
0K
First Participant, First Injection
Last Participant, Last Injection
End of the 90-Day Follow-Up
Alpha
Beta
Delta
Omicron
Mar 22, 20
Jun 14, 20
Sep 6, 20
Nov 29, 20
Feb 21, 21
May 16, 21
Aug 8, 21
Oct 31, 21
Jan 23, 22
Apr 17, 22
Confirmed Cases
Deaths

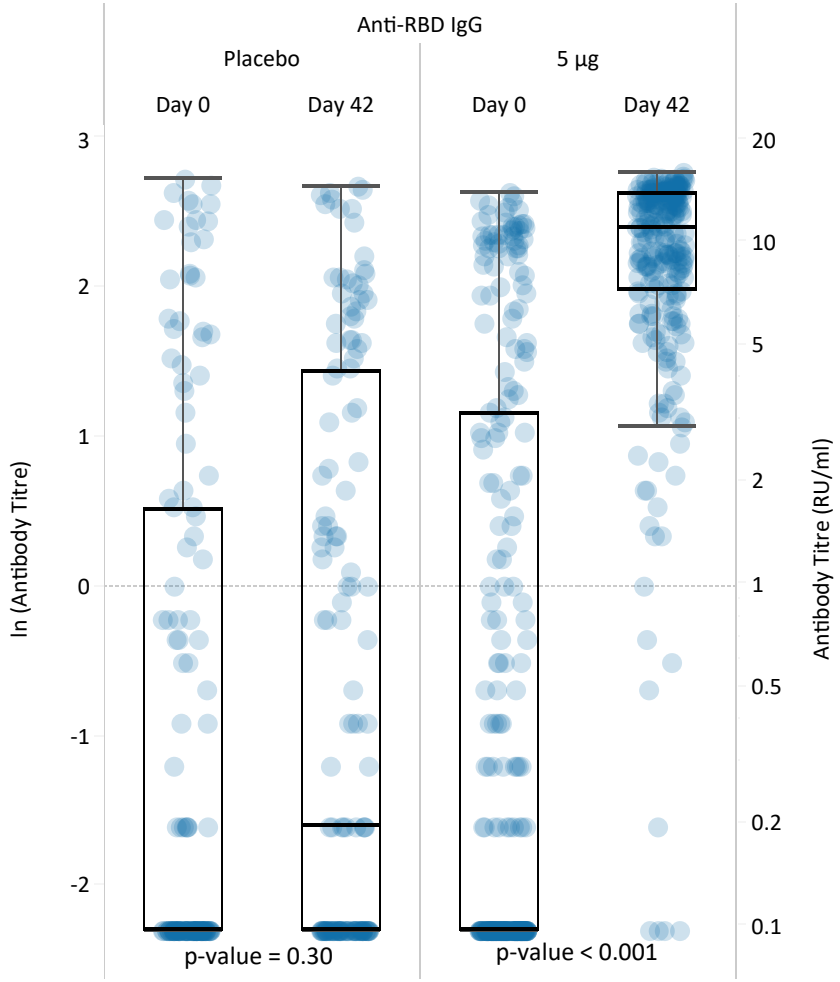


*Death occurred within 14 days after the second injection; thus not included in the efficacy population

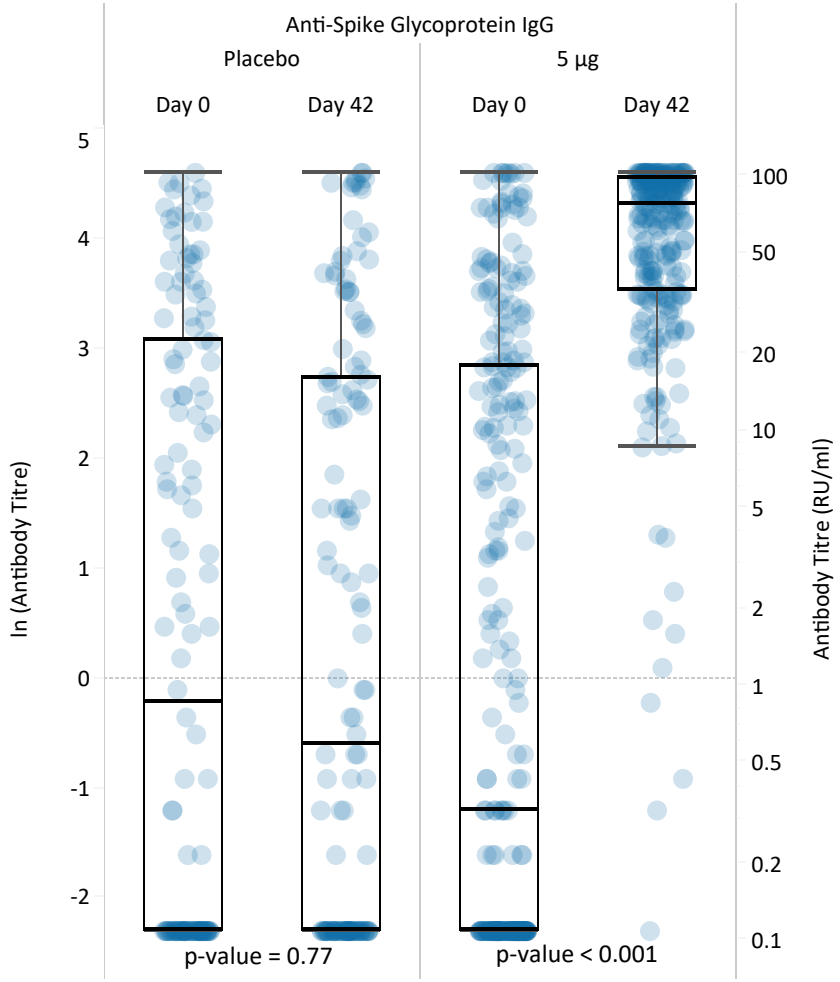
Phase III – Neutralising Antibody



Phase III – Anti-RBD IgG



Phase III – Anti-Spike Glycoprotein IgG



Supplemental appendix 1

STUDY PROTOCOL

Appendix to:

Efficacy and Safety of an Inactivated Virus-Particle Vaccine for SARS-CoV-2, BIV1-CovIran: Findings from a Randomised, Placebo-Controlled, Double-Blind, Multicenter Phase III Clinical Trial amid the Delta Variant Peak

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Table of Contents

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Trial Registration Data Set

Data category	Information
Primary registry and trial identifying number	IRCT20201202049567N3
Date of registration in primary registry	13 March, 2021
Secondary identifying numbers	N/A
Primary sponsor	Shifa-Pharmed Industrial Group.
Secondary sponsor(s)	N/A
Contact for public queries	Mohammadreza Salehi [salehi.mohamad3@gmail.com]
Contact for scientific queries	Minoo Mohraz, MD [minoomohraz@ams.ac.ir]
Public title	The safety and efficacy of a COVID-19 inactivated vaccine
Scientific title	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
Countries of recruitment	Iran
Health condition(s) or problem(s) studied	COVID-19
Intervention	Active comparator: Vials containing 5 micrograms of inactivated whole virus particle vaccine against SARS-CoV-2 with aluminium hydroxide adjuvant
	Placebo comparator: Matching vials containing no active ingredients with aluminium hydroxide adjuvant

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Key inclusion and exclusion criteria	Ages eligible for study: 18 to 75 years old Sexes eligible for study: both Accepts healthy volunteers: Yes
	Inclusion criteria: • Volunteers aged 18 to 75 with a generally healthy condition based on medical history, clinical laboratory results, vital sign measurements, and physical examination in the screening session. • All eligible volunteers must fully understand the study processes, the explanations of the facilitators, and the contents of the informed consent form. • Female participants, unless menopause, will be required to have a negative pregnancy test at screening or vaccination. • All participants of reproductive age will agree to use effective methods of contraception during the study. • All participants will agree not to donate blood, blood products, or bone marrow from the start of the vaccine candidate until three months after receiving the last dose.
	Exclusion criteria: • Confirmed, suspected, or asymptomatic COVID-19 detected by RT-PCR at baseline, • 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, and shortness of breath during the 14 days before vaccination, • History of severe allergy or allergic reactions to inactivated vaccine components, • Currently known cases of tuberculosis, hepatitis B, or hepatitis C,

	• History of coagulopathy, • History of splenectomy, • Any history of uncontrolled chronic conditions, including uncontrolled blood pressure (systolic and diastolic blood pressure above 140 and 90 mmHg, respectively), diabetes mellitus, severe and chronic heart, kidney, liver, neurological or pulmonary diseases retrieved by medical examinations or 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 will be able to attend the study like other healthy individuals. • Acute illness or exacerbation of chronic diseases in the last seven days, • Any malignancy, immune deficiency disease, Human Immunodeficiency Virus (HIV), lymphoma, leukaemia, or other autoimmune diseases, • Receiving immunomodulatory or immunosuppressive therapies for at least 14 consecutive days in the last three months or having a plan to receive over the next year (in the case of corticosteroids, a dose equivalent to more than 20mg per day of prednisolone for more than seven days) during the last three months, not including topical and inhaled use, • Immunosuppressants, including chemotherapy drugs, drugs for the treatment of multiple sclerosis, 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,

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	Levamisole, Isoprinosine, Thymosin, and any other medication with evidence on immunomodulation, • Receiving any live vaccines one month before injection 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, • History of receiving immunoglobulins or blood products three months before injection or planning to receive over the next year, • History of receiving any investigational drugs six months before injection, • Planning 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, • Any other circumstances other than those mentioned above that the Principal Investigator deems inappropriate for participation in the clinical trial.
Study type	Interventional
	Allocation: randomized intervention model. Parallel assignment masking: double-blind (subject, caregiver, investigator, outcomes assessor), multicenter
	Primary purpose: COVID-19 prevention
	Phase III
Date of the first enrolment	May 2021

Target sample size	A total of 20,000 participants are randomly assigned to intervention/placebo groups with a ratio of 2:1.
Recruitment status	Recruitment completed
Primary outcome(s)	(A) Comparison of the efficacy of BIV1-CovIran vaccine candidate and placebo from 14 to 180 days after receiving the last dose of vaccine candidate/placebo for non-severe, severe, critical cases and deaths due to COVID-19, (B) The number of non-severe, severe, and critical cases of COVID-19 from 14 to 180 days after receiving the last dose of the vaccine candidate, (C) Prevention of death due to COVID-19 within 180 and 360 days after receiving the last dose of the vaccine candidate.
Key secondary outcomes	(A) Comparison of the efficacy of BIV1-CovIran vaccine and placebo from 181 to 360 days after receiving the last dose of vaccine candidate/placebo for non-severe, severe, critical cases and deaths due to COVID-19, (B) The number of non-severe, severe, and critical cases of COVID-19 from 181 to 360 days after receiving the last dose of vaccine candidate/placebo, (C) The occurrence of any immediate reactions (local and systemic) after each administration (days 0, 28; up to 30 minutes), (D) 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, (E) 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, (E) The occurrence of any solicited adverse events (AEs) up to 7 days after each administration,

	(F) The occurrence of any unsolicited AEs from 8 to 28 days after each administration, (G) The occurrence of any SAEs after each administration up to 360 days after the second injection. (H) The geometric mean titers (GMT), geometric mean ratios (GMR) of antibodies against SARS-CoV-2 (neutralizing, anti-Receptor Binding Domain (anti-RBD), and anti-spike glycoprotein (anti-S) antibodies) and the seroconversion rates at baseline and 14 days after second injection.

Introduction

Background and rationale

Effective and safe vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could be a potential pathway for controlling the coronavirus disease-2019 (COVID-19) pandemic if distributed promptly and just [1] As of 15 October 2021, 320 vaccines were being studied, among which 126 vaccines have been tested on humans in clinical trials [2]. Several COVID-19 vaccines have shown promising results in phase III clinical trials so far, and vaccinations began in early 2021 [3,4]. As of May 2022, World Health Organisation (WHO) has authorized emergency use for eleven vaccines and continues to evaluate additional proposals [5].

In Iran, COVID-19 took a heavy toll on the healthcare system, which has resulted in 6.13 million confirmed cases and 130 thousand deaths[6]. From the beginning of the COVID-19 pandemic in Iran, there was limited access to life-saving medicines and equipment, which resulted in a significant burden on the Iranian healthcare system[7]. Due to suboptimal management and supply challenges, only 3.5% of the Iranian population were fully vaccinated nine months after introducing the first COVID-19 vaccine [8]. Therefore, prompt manufacturing and administration of a safe homegrown anti-SARS-CoV-2 vaccine seem imperative and crucial.

Among the vaccine platforms, inactivated vaccines have been extensively used for various diseases such as influenza, hepatitis A, polio, and rabies [9]. Thus, they have a well-studied efficacy and safety profile and established evaluation and quality control methodologies [10]. They could also be easily stored and shipped at 2 °C to 8 °C, which would make them suitable for low-income and low-middle income countries [11,12]

BIV1-CovIran [13] is an inactivated whole virus particle vaccine manufactured by Shifa Pharmed Industrial Group. The SARS-CoV-2 virus was sequenced and cultured using a Vero cell manufacturing platform in a biosafety level 3 (BSL-3) facility. 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). Each dose of vaccine included a maximum of 500 µg of Alhydrogel. Further details about vaccine production are presented elsewhere [14]. The placebo solution contained the same amount of Alhydrogel, diluted by phosphate-buffered saline. Vaccine and placebo vials were stored at 2-8°C.

BIV1-CovIran has shown to be generally safe and has induced antibody responses in adults 18 years and older in phase 1/2 trials [13]. However, its efficacy has yet to be demonstrated and its safety needs to be evaluated in a larger sample size. The study presents the protocol of a randomized, placebo-controlled, double-blind, multicenter phase III clinical trial for the BIV1-CovIran vaccine candidate.

Objectives

Primary objectives

- Evaluation of the BIV1-CovIran vaccine candidate efficacy against non-severe COVID-19 infection among 18-75-year participants from 14 days up to 180 days after the second injection,
- Evaluation of the BIV1-CovIran vaccine candidate efficacy against severe COVID-19 infection among 18-75-year participants from 14 days up to 180 days after the second injection,
- Evaluation of the BIV1-CovIran vaccine candidate efficacy against critical COVID-19 infection due to COVID-19 infection among 18-75-year participants from 14 days up to 180 days after the second injection,
- Evaluation of the BIV1-CovIran vaccine candidate efficacy against deaths due to COVID-19 infection among 18-75-year participants from 14 days up to 180 days after the second injection.

Secondary objectives

- Evaluation of the BIV1-CovIran vaccine candidate efficacy against non-severe, severe, critical infection and deaths due to COVID-19 infection among 18-75-year participants from 181 days up to 360 days after the second injection.
- Evaluation of the BIV1-CovIran vaccine candidate safety among 18-75-year participants,
- Exploratory evaluation of the BIV1-CovIran vaccine candidate immunogenicity among 18-75-year participants.
- Evaluation of BIV1-CovIran vaccine candidate/placebo efficacy after receiving an intervention dose from day 1 to before the second intervention.

Trial design

This randomized, placebo-controlled, double-blind, multicenter Phase III clinical trial will be conducted to evaluate the efficacy and the safety of an inactivated vaccine candidate, BIV1-CovIran, for SARS-CoV-2 during the COVID-19 pandemic in six cities of Iran. In addition, to further evaluate the immunogenicity of the vaccine candidate, the humoral response of a subsample of 400 participants will be assessed in Phase III. The study will abide by the declaration of Helsinki, Good Clinical Practice (GCP), and the Iran GCP (IR-GCP) as the local regulator. Face-to-face visits will be performed on screening day, the first injection day, the second injection day and two weeks after each injection in the predetermined centres. In addition, the participant will be assessed in-person in case of any need for clinical evaluation. All participants will be followed up via over-the-telephone interview in a 14-day interval from the day of injection to one year after the second injection and will be assessed for Adverse Events (AEs) and COVID-19 outcomes by trained nurses and physicians. In addition, all participants will be required to complete an electronic reactogenicity diary book regarding any possible COVID-19 symptoms. All Phase III participants will be capable of contacting and in-person 24/7 study centres, also providing video call or file-sharing features, should they have any concerns or need medical attention. Upon onset of any suspicious COVID-19 symptoms (Table 1) [15], a nasopharyngeal specimen would be obtained, and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) would be performed at the clinical trial site. In cases of negative RT-PCR tests, participants underwent a further RT-PCR test after 48 hours unless their symptoms regressed. COVID-19 severity status was categorized as non-severe, severe, and critical based on the diagnosis scheme in Iran and the WHO classification (Table 2). For further immunogenicity assessments, blood samples of a subset of 400 participants will be obtained on days 0 and 42 (14 days after the second injection) and will be assessed for humoral immunity responses (Figure 1).

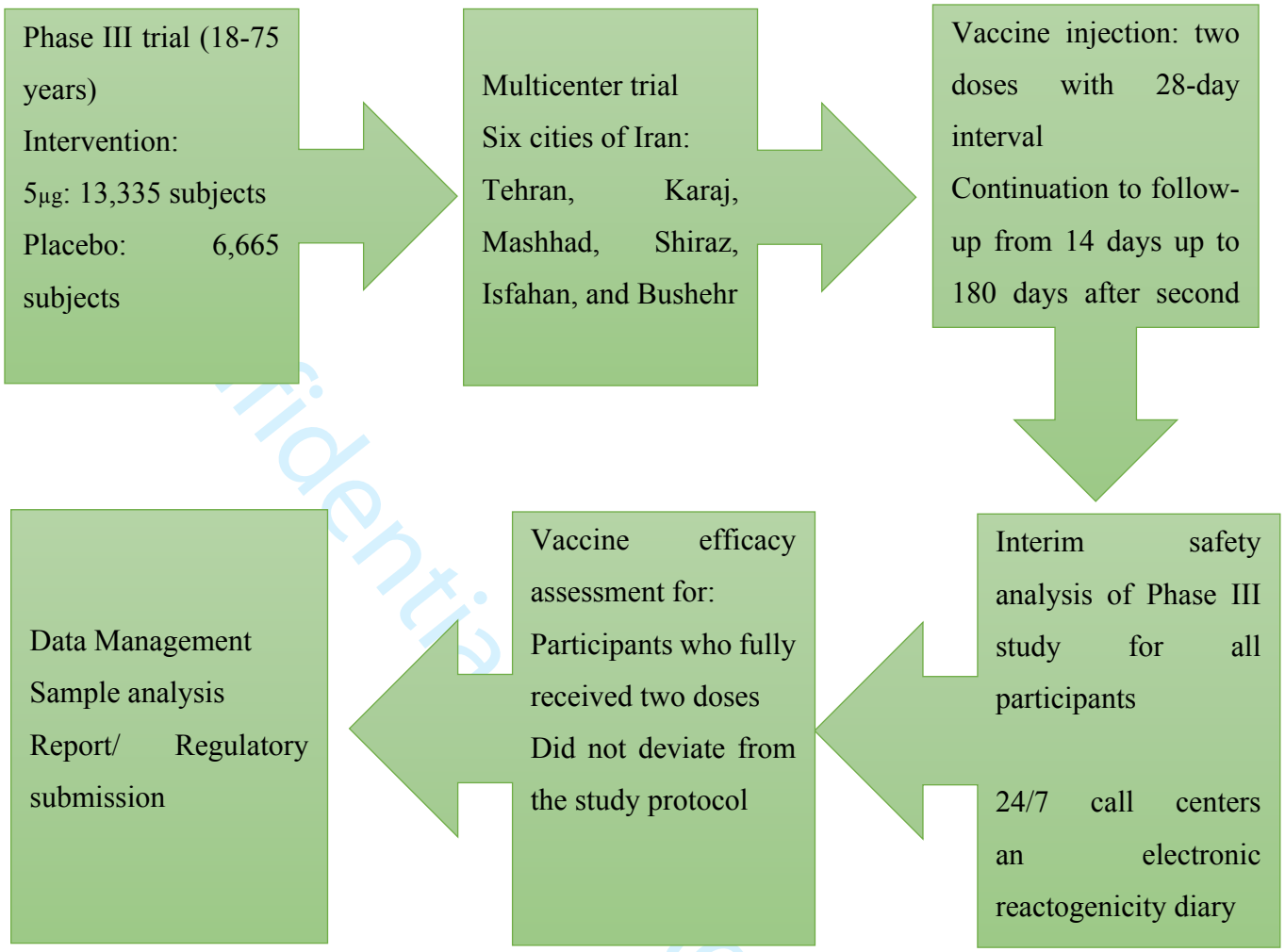

Figure 1. Phase III clinical trial study design

Table 1. Suspected COVID-19 symptoms for considering RT-PCR among participants and case definition criteria

Symptom	Minimum time of persistence
Fever	24 hours
New-onset cough/ Worsening of the previous cough	24 hours
Dyspnea	24 hours
Chills	48 hours
Rhinorrhea/ Congestion	48 hours
Sore throat	48 hours
Myalgia	48 hours
Headache	48 hours
New-onset loss of smell/taste	48 hours
Diarrhea	48 hours

Nausea/vomiting	48 hours

Case definition criteria

At least 2 of the following symptoms: fever (axillary temperature ≥37.5 °C), chills, sore throat, stuffy nose, myalgia, fatigue, headache, nausea or vomiting, or diarrhoea

At least one respiratory sign or symptom (including cough, shortness of breath), new olfactory or taste disorder, radiographic evidence of COVID-19 like pneumonia.

Table 2. World Health Organization classification for COVID-19 severity

Grade	Definition
Non-severe	Patients with positive RT-PCR test, AND Absence of any criteria for severe or critical COVID-19
Severe	Oxygen saturation < 90% on room air, Signs of severe respiratory distress (accessory muscle use, inability to complete full sentences, respiratory rate > 30 breaths per minute), in addition to the signs of pneumonia. *
Critical	Defined by the criteria for acute respiratory distress syndrome (ARDS), sepsis, septic shock, or other conditions that would normally require the provision of life-sustaining therapies such as mechanical ventilation (invasive or non-invasive) or vasopressor therapy.
Death	Passed away patients with positive RT-PCR

* Patients with RT-PCR, who were treated with Remdesivir without hospitalization according to National COVID-19 guideline were considered as severe cases.

Methods and Analysis: Participants, interventions, and outcomes

Study setting

The first vaccine candidate/placebo injection of the first participant in Phase III will occur in May 2021, and the last dose is planned to be injected on 20 July. This study will be performed with the participation of 20,000 eligible volunteers aged 18-75 years in six cities of Iran, including Tehran, Karaj, Mashhad, Shiraz, Isfahan, and Bushehr. The number of samples for each city will be determined based on the city population as estimated 8,500 participants for Tehran, 2,000 for Karaj, 3,500 for Mashhad, 2,500 for Shiraz, 2,500 for Isfahan and 1,000 for Bushehr.

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Eligibility criteria

All volunteers aged 18 to 75 years old with a generally healthy condition will be included in the study based on medical history, clinical laboratory results, vital sign measurements, and physical examination in the screening session. All eligible volunteers must fully understand the study processes, the explanations of the facilitators, and the contents of the informed consent form. Female participants, unless menopause, will be required to have a negative pregnancy test at screening or vaccination. All participants of reproductive age will agree to use effective methods of contraception during the study. Moreover, they will decide not to donate blood, blood products, or bone marrow from the start day of the trial until three months after receiving the last dose.

Exclusion criteria

The exclusion criteria for participants in Phase III will be as follows:

- Confirmed, suspected, or asymptomatic COVID-19 detected by RT-PCR at baseline,
- 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, and shortness of breath during the 14 days before vaccination,
- Receiving Anti-SARS-Cov-2 vaccines of any type and at any time before the trial,
- History of severe allergy or allergic reactions to inactivated vaccine components,
- Currently known cases of tuberculosis, hepatitis B, or hepatitis C,
- History of coagulopathy,
- History of splenectomy,
- Any history of uncontrolled chronic conditions, including uncontrolled blood pressure (systolic and diastolic blood pressure above 140 and 90 mmHg, respectively), diabetes mellitus, severe and chronic heart, kidney, liver, neurological or pulmonary diseases retrieved by medical examinations or 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 will be able to attend the study like other healthy individuals.
- Acute illness or exacerbation of chronic disease in the last seven days,

- Any malignancy, immune deficiency disease, HIV, lymphoma, leukaemia, or other autoimmune disorders,
- Receiving immunomodulatory or immunosuppressive therapies for at least 14 consecutive days in the last three months or having a plan to receive over the next year (in the case of corticosteroids, a dose equivalent to more than 20mg per day of prednisolone for more than seven days) during the last three months, not including topical and inhaled use,
- Immunosuppressants including chemotherapy drugs, drugs for the treatment of multiple sclerosis, 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, Isoprinosine, Thymosin, and any other medication with evidence on immunomodulation,
- Receiving any live vaccines one month before injection 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,
- History of receiving immunoglobulins or blood products three months before injection or planning to receive over the next year,
- History of receiving any investigational drugs six months before injection,
- Planning to participate in another clinical trial or extension studies 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,
- Any other circumstances other than those mentioned above that the Principal Investigator (PI) deems inappropriate for participation in the clinical trial.

Second dose injection exclusion criteria

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

- Positive pregnancy test (Beta-HCG) before the second injection,

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- Presenting with a temperature over 39^{0}C over three days or any severe allergic reaction after the first injection,
- Reporting any Serious Adverse Events (SAE) after the first injection,
- Receiving immunoglobulin or steroidal hormones (oral or intravenous) up to two weeks before the second injection.

Interventions

Description: study vaccine candidate/placebo

BIV1-CovIran is an inactivated whole virus particle vaccine candidate 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. Viral particles were inactivated with β-propiolactone. After purification, the inactivated virus particles were sterilized with filtration and formulated with Al-hydrogel as an adjuvant.

Further details about vaccine production are presented elsewhere [14]. The placebo solution contained the same aluminium hydroxide adjuvant. Vaccine and placebo vials will be stored at 2-8°C.

All participants in Phase III will be randomly assigned to one of the following arms:

Arm 1: BIV1-CovIran vaccine candidate

Dose: 0.5 mL, 5µg of the vaccine candidate

Route and mode of injection: Intramuscular (Deltoid muscle)

Dosage schedule: days 0 and 28

Arm 2: Placebo

Dose: 0.5 mL

Route and mode of injection: Intramuscular (Deltoid muscle)

Dosage schedule: days 0 and 28

Description: vaccine injection

Vaccine candidate/ placebo vials, labelled with random sequences, will be stored in a refrigerator equipped with a data logger at 2-8°C. The maximum time between removing the vaccine from the heat-insulating containers and completing the injection process will be 30 minutes. Vials with abnormal hue, smell, dosage, or damaged vials will be discarded. The vaccinator will double-check

the participants' identity and informed consent before injection. In addition, the vials will be kept in room temperature for two minutes before injection. The 0.5 millilitres of the vaccine candidate/ placebo will be injected into the Deltoid muscle of the non-dominant arm. All participants will stay at the vaccination site for 30 minutes after the injection to assess the immediate adverse events.

Modification

Early discontinuation

Following any of the following conditions, the clinical trial will be terminated.

- Any suspicion of the potential safety risks or the quality issues of vaccine candidate during the trial that requires a thorough review or revision by the study sponsor or the Data Safety Monitoring Board (DSMB),
- Any suspected morality issues by local regulators, including the National Research Ethics committee and Iran Food and Drug Administration (IFDA)
- Termination requests by the non-regulatory authorities.

Study suspension

It will be planned to suspend the trial following each of the conditions described below. It will be required to hold a joint meeting with the outcome assessor, sponsor, DSMB, and the ethics committee regarding the study termination. It is worth mentioning that the DSMB meeting will be required in each of the following cases.

- Occurrence of grade four AE in placebo or intervention group,
- Occurrence of suspected unexpected SAEs related to the procedure in placebo or intervention group,
- Occurrence of grade three AE among more the 15% of participants regardless to their subgroup or injection dosage, and
- DSMB decision about a high potential risk to safety.

It is worth mentioning that the AEs' grading will be based on the FDA Guidelines for Toxicity Rating in Healthy Individuals Participating in Vaccine Studies.

Concomitant care

The drug history of the participants will be obtained during every visit and recorded in electronic Case Report Forms (eCRF). Medications will be not withheld if required for a participant's medical care. The immunosuppressant or immune modifying medication including Azathioprine,

Cyclosporin, Interferon, G-CSF, Tacrolimus, Everolimus, Sirolimus, high-dose systemic corticosteroids, immunoglobulin or blood derivatives are prohibited to all study participants from the time of informed consent until 21 days after the second dose injection unless the participant is in need for drugs mentioned above. In these cases, the participant will be excluded from the study. All participants will be trained to record their drug history in the eCRFs. In addition, 30 days after the intervention, the participants will be allowed to use all live, attenuated, and inactivated vaccines. Emergency vaccinations, including the rabies vaccine, will not be prohibited.

Outcomes

Primary and secondary endpoints of the BIV1-CovIran vaccine candidate Phase III clinical trial is presented in Table 3.

Table 3. Primary and secondary endpoints of BIV1-CovIran vaccine candidate Phase III clinical trial

Primary endpoints		
Efficacy endpoint		
(A) Comparison of the efficacy of BIV1-CovIran vaccine candidate and placebo from 14 to 180 days after receiving the last dose of vaccine candidate/placebo for non-severe, severe, critical cases and deaths due to COVID-19, (B) The number of non-severe, severe, and critical cases of COVID-19 from 14 to 180 days after receiving the last dose of the vaccine candidate, (C) Prevention of death due to COVID-19 within 14 and 180 days after receiving the last dose of vaccine candidate.		
Secondary endpoints		
Efficacy endpoints	**Safety endpoints**	**Immunogenicity endpoints**
(A) Comparison of the efficacy of BIV1-CovIran vaccine and placebo from 181 to 360 days after receiving the last dose of vaccine candidate/placebo for non-severe,	(A) The occurrence of any immediate reactions (local and systemic) after each administration (days 0, 28; up to 30 minutes), (B) The occurrence of any local reactions at the injection site	(A) The geometric mean titers (GMT), geometric mean ratios (GMR) of antibodies against SARS-CoV-2 (neutralizing, anti- Receptor Binding Domain (anti-RBD), and anti-spike glycoprotein

severe, critical cases and deaths due to COVID-19, (B) The number of non-severe, severe, and critical cases of COVID-19 from 181 to 360 days after receiving the last dose of vaccine candidate/placebo.	after each administration (including pain, itching, swelling, inflammation, redness, skin rash, and irritation up to 7 days after administration, (C) 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, (D) The occurrence of any solicited adverse events (AEs) up to 7 days after each administration, (E) The occurrence of any unsolicited AEs from 8 to 28 days after each administration, (F) The occurrence of any SAEs after each administration	(anti-S) antibodies) and the seroconversion rates at baseline and 14 days after the second injection.

Participant timeline

In Phase III, a face-to-face interview will be held – during the screening session, first injection day, two weeks after the first injection, and second injection day. Over-the-telephone follow-up visits will be held every 14 days after the injection until day 360 (Table 4).

Table 4. Phase III procedures' timeline

Parameters	Visit 1 Screening	Visit 2 Injection	Visit 3	Visit 4 Follow-up	Visit 9
Days	**-7 to -1**	**0**	**28±3**	**42±3**	**388±7**

Medical history	×				
Inclusion/ exclusion criteria	×		×		
Informed consent	×	×			
Physical examination	×				
Demography data	×				
Randomization		×			
Drug history		×	×	×	×
Inoculation		×	×		
RT-PCR test	×				
Blood sampling	×				
Over-the-phone follow-up				×	×
Adverse Events Assessment		×	×	×	×

Screening (-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 neutralizing antibody (IgG) for COVID-19 screening will be gathered from all eligible volunteers, and they will be enrolled.

Randomization and first administration (Day 0)

All participants in Phase III 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 with a 2:1 ratio. In visit one, a vaccine dose or placebo will be administered. Following the first dose administration, any immediate adverse events will be recorded. All participants will be educated regarding electronic eCRF completion.

Second dose administration (Day 28+3)

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 recorded. During this visit, the second vaccine candidate or placebo dose will be administered to participants who do not meet the second dose injection exclusion criteria. Following the first dose administration, any immediate adverse events will be recorded.

Follow-up visits (Day 42 to Day 360)

All participants will be followed up via over-the-telephone visits by nurses regarding COVID-19 symptoms on days 42, 56, 70, 98, 112, 126, 140, 154, 168, 182, 196, 210, 224, 238, 252, 266, 280, 294, 308, 322, 336, 350, 364, 378, and 388. Participants with suspected symptoms will be referred

to RT-PCR laboratory centres and medical examinations. In addition, all AEs regarding vaccine candidate/placebo injection will be recorded on eCRFs. Blood samples will be obtained on day 42 from a subgroup of 400 participants who were selected for humoral response investigation.

Sample size

In order to estimate the accurate sample size for vaccine efficacy evaluation, the study should meet the WHO recommendation of achieving 150 COVID-19 cases across the vaccine/placebo groups, an ultimate efficacy of 60% with a 95% confidence interval lower limit of no less than 30%. Therefore, by assuming vaccine efficacy of 0.6 with a 95% confidence interval larger than 0.3, the vaccine candidate will be considered efficacious. Considering the current trend of the epidemic in Iran and the cumulative number of confirmed, the monthly incidence rate of COVID-19 among the placebo group was assumed to be 1%. The allocation ratio of vaccine candidate and placebo groups was 2:1, and the dropout rate was considered as 10%. Considering the multicenter study site distribution, 20,000 cases were planned, with 13,350 participants in the intervention group.

Recruitment

Invitations to participate will be shared on mass and social media platforms, and all volunteers will be provided with contact information. Eligible volunteers will be reached by phone calls from study physicians and then receive detailed explanations about the study protocol and procedures. Willing and eligible volunteers will be invited to an in-person appointment at a determined time and date. Following the in-person session, the volunteers will be provided written informed consent and undergo clinical and paraclinical evaluations.

Methods and Analysis: Assignment of interventions

Allocation: Sequence generation

During Phase III, the participants will be randomized into intervention and placebo groups with a 2:1 ratio, including 13,350 volunteers in the intervention group and 6,650 in the placebo group. Using an electronic tool [16], block sequences with sizes 3 and 6 will be produced according to the number of sample volumes. In Phase III, the number of samples of each city will be fundamentally proportionate to the city population. In addition, considering the importance of matching the samples with the population age pyramid of the country, the randomization will be stratified and will be performed in two layers of 18-50 and 51-75 age groups with a ratio 4:1, respectively.

Allocation: Implementation

Following the randomization, a unique code will be allocated to each participant. The code will have eight characters, including the first two letters of the first name, the first two letters of the last name, and four generated characters from the random chain. All eCRFs will be filled similarly using the proprietary code throughout the study. All procedures during Phase III will be performed using the participant code, and the identification information will remain confidential by PI.

Allocation concealment mechanism

Vials will be packaged separately and have an identification number. Vaccine and placebo boxes are offered in exactly similar appearance, which will blind the participants, investigator, and outcome assessors. Participants' allocated code and vaccination date will be recorded on both outer vial packaging box trial master sheets following the vaccination. Throughout the study, all outer packing boxes will be maintained.

All laboratory assessments and results will be reported using participants' unique allocated code. Participants, outcome assessors, study coordinators, and other site staff will be blinded. Only the academic Contract Research Organisation (CRO) affiliated with the Clinical Trial Center, Tehran University of Medical Sciences, Tehran, Iran, responsible for labelling and data administration, will be unblinded at the study site.

Blinding (masking)

In order to carry out the blinding process, vaccine vials and placebo will be offered in precisely the same appearance, label, and unique identification code, which will guarantee the participants, researchers, and outcome assessors' blindness. After the vaccine administration, the unique code of the participant and the date of vaccination will be written in the outer packaging box, and the label will be recorded on the main sheet. The study personnel will check all information before injection. During the study, all packages will be archived and maintained. All participants' data or laboratory assessments will be collected and labelled by their unique randomization code.

Blinding (masking): emergency unblinding

In cases of any emergency events, including serious adverse events (SAEs), the query of emergency decoding and unblinding will be urgently requested by the PI. The emergency decoding will be performed using an online system, the log of which will be stored. Nevertheless, only one authorized investigator will be allowed to emergency decoding and unblinding. The history of all

decoding and unblinding, including the reason for the request, informing the sponsor, ethics committee, clinical studies monitoring office, and required filled forms, will remain in the system archive.

Methods and Analysis: Data collection and analysis

Data collection

Vital signs (sitting blood pressure, pulse rate, respiratory rate and temperature) will be measured after 5 minutes of rest. Blood pressure and heart rate will be measured using a standard calibrated blood pressure measuring device. Participants should be seated on a chair, back supported, and arms bared free of restrictions. Measurements should be taken on the same arm at each visit. Heart rate should be measured at approximately the same time as BP for a minimum of 30 seconds. Measurement of body temperature will be collected using the tympanic methods, and the same method should be used consistently throughout the study. Bodyweight will be obtained with the patient wearing undergarments or very light clothing, no shoes, and an empty bladder. The use of calibrated balance scales is recommended. Height should be measured at screening. Self-reported weights and heights are not acceptable.

Participants will be required to complete an electronic daily reactogenicity diary book using a mobile application designed for this clinical trial, which also enables voice and video contact with the trial 24/7 call centres and gathering the data regarding participants' symptoms and AEs. All participants will be trained regarding the installation and work with the application. Participants in Phase III will be asked to monitor and record local reactions, systemic events, and history of antipyretic medication use following the injection in the mobile application. Data from electronic eCRFs will be gathered and investigated using standard statistical methods. Safety data will be investigated using the frequency (percent) of adverse events incidence. Efficacy analysis will be performed by standard vaccine efficacy calculation methods. Geometric mean titres will be used for exploratory immunogenicity analysis. The impact of all outliers in data on study findings will be assessed and announced in the final report.

Blood samples will be collected following the physical assessments before injection. Blood samples in the first day will be used to assess baseline COVID-19 IgG, IgM, hepatitis B, hepatitis C, and humoral antibody (neutralizing, anti-RBD domain and anti-spike glycoprotein) levels.

Humoral assessment will be performed 14 days after the second injection. COVID-19 suspected cases will undergo nasopharyngeal swab for SARS-Cov-2 RT-PCR.

Data management

The PI will be responsible for maintaining all essential documentation related to the participants over time. Following the request of the data management team and sponsor approval, eCRFs will be submitted to the Data Management Center at the end of the study. A copy of the documentation will be kept in the study centres in each trial city. It is worth mentioning that the documentation transfer process will comply with all security and safety principles. Supervision of the correct execution of all processes will be the responsibility of the PI.

Data quality control

Data quality control will ensure that all participation processes will align with the ethical principles and that valid data will be gathered thoroughly. In addition, immediate identification of issues and problems to provide appropriate operational and corrective plans will be another equally corroborating objective of data quality control.

Quality control will be performed through the following steps. Firstly, a meeting will be held before the study starts with the participation of the executive team and investigators at the study site. The study protocol will be reviewed in the meeting, and the implementation process re-explained. Moreover, the executive team visited the volunteer entry sites and performed role-playing to ensure the accuracy of the workflow. Secondly, quality control will be performed daily with thorough documentation and recording.

Quality control will be performed at two levels:

- **eCRF completion:** All eCRFs will be reviewed by the executive team at the study. The executive team at the study site will be responsible for providing an action plan to improve the quality of eCRFs.
- **Central database:** The following parameters will be evaluated daily in addition to the standard measures available for data management:
    - Checking the box related to the informed consent form
    - Missing data management for the key variables of the study
    - Completing the questionnaire form of the participant

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Statistics

Analysis principals

All data collected from Phase III will be analyzed based on two analysis populations: the safety population and the efficacy population.

- **Safety population:** Participants enrolled in the study and received at least one dose of the study drug.
- **Efficacy population:** Participants who abided by the protocol without significant deviations from the study protocol basics for at least 180 days after the second injection.

Analysis set

The analysis would be performed using the per-protocol set (PPS) approach. Participants who signed the informed consent and enrolled in the study were defined as the intention-to-treat (ITT) population. The PPS population describes as being without any significant deviations from study protocol basics.

Study profile

The final statistical report will include all participants who sign the informed consent form. The flowchart recommended by Consolidated Standards of Reporting Trials (CONSORT) will be used to show the presence of participants from the moment of admission to the clinical trial (screening and review of criteria) to the end of the study (evaluation of study outcomes). The number (percentage) of participants in each group will be reported for the PPS population, and the reasons for withdrawal or significant deviations from the protocol will be gathered.

Sociodemographic and health status of participants

To identify any statistical difference between the two groups in terms of demography and health status, descriptive statistics, including central tendency measures including mean, and median, and dispersion indices including standard deviation and interquartile ranges, will be calculated. Frequencies and percentages will be considered for continuous variables.

Efficacy analysis

The efficacy assessment will be performed on the efficacy population. The vaccine's efficacy will be estimated using Poisson Regression Model as a 1-adjusted relative risk between vaccinated to

placebo recipients. The efficacy will be calculated against COVID-19 non-severe, severe, critical cases, and deaths due to COVID-19.

$$Vaccine\ efficacy\ (\%) = \left(1 - \frac{\text{Incidence density of intervention group}}{Incidence\ density\ of\ placebo\ group}\right) \times 100$$

$$Incidence\ density = \left(\frac{\#\ confirmed\ cases\ during\ the\ effective\ follow-up}{\#observation\ years\ of\ all\ vaccinated\ participants}\right) \times 100$$

The observation years will be calculated by the termination of follow-up once the participants were positive for COVID-19 RT-PCR or withdrew from the study.

Safety analysis

The safety assessment will be performed on the safety population regarding AEs. Numbers and percentages (Clopper-Pearson method) of participants with local and systemic AEs will be reported based on the Food and Drug Administration (FDA) toxicity grading scale using a 95% Clopper-Pearson confidence interval. The AEs will be defined by Medical Dictionary for Regulatory Activities (MedDRA), version 23.1.

Immunogenicity analysis

Immunogenicity assessments will be performed among all participants who received at least one vaccine candidate/placebo dosage throughout the study. The humoral response will be assessed through geometric mean titers (GMT), geometric mean ratios (GMR) of antibodies against SARS-CoV-2, and seroconversion rate. Neutralizing, anti-RBD, and anti-S antibodies will be measured using Enzyme-Linked ImmunoSorbent Assay (ELISA) kits.

Methods and Analysis: Monitoring

Data monitoring: formal committee

The data safety monitoring board (DSMB), as an independent committee during predetermined sessions, which aims to ensure the safety of the research product (Safety review) and the progress process, periodically will collect and review the safety and efficacy of the product. The committee will decide whether to continue, suspend, or terminate the clinical trial throughout the study. In addition, the meetings of this committee might increase once severe or serious adverse events happen. The meetings of this committee will be held online or in person with the participation of the sponsor's representative, the Principal Investigator (PI), the independent members of the committee and the regulatory representatives (Table 5).

Table 5. Timeline of the Data Safety and Monitoring Board meetings during Phase III

Meeting	Time	The main objective
1	After the completion of the 90-day follow-up of 30% of Phase III participants	1- Reviewing the study process and announcing the considerations related to continuing the study, 2- Reviewing the safety and efficacy of the vaccine candidate among participants.
2	After the completion of the 90-day follow-up of 50% of Phase III participants	1- Reviewing of the study process and announcement of the considerations related to continuing the study, 2- Reviewing the safety and efficacy of the vaccine candidate among participants.
3	After the completion of the 90-day follow-up of 70% of Phase III participants	1- Reviewing of the study process and announcement of the considerations related to continuing the study, 2- Reviewing the safety and efficacy of the vaccine candidate among participants.
4	After the completion of the 90-day follow-up of 100% of Phase III participants	1- Reviewing of the study process and announcement of the considerations related to continuing the study, 2- Reviewing the safety and efficacy of the vaccine candidate among participants.

Data monitoring: interim analysis

The interim analysis will be performed following the 6-month follow-up after the first injection for half of the Phase III participants. A 6-month follow-up of half of the participants, evidence regarding vaccine efficacy of at least 60%, and the occurrence of at least five critical COVID-19 outcomes or death due to COVID-19 among the placebo group will be required conditions for the authorization of emergency use by the National Research Ethics Committee.

Harms

Throughout the study, the potential harms of the intervention will be categorized as AEs, including local and systemic solicited (Day 0 to 7 after vaccination) and unsolicited (Day 8 to 28 after vaccination) AEs; SAEs; vital sign abnormalities; and physical examination findings.

Adverse Events

An AE will be 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. The AEs will be reported in two disparate categories: 1) systemic and local

AEs, and 2) solicited and unsolicited AEs. The PI will check the Relatedness/causality and severity grading of AEs based on the WHO causality assessment.

Serious AEs

Any consequence that results in death, risk of death, hospitalization, persistent or significant disability, surgical intervention or medical intervention that leads to or causes malformation or congenital malformations will be considered SAEs.

Grading

Grading will be based on the FDA Guidelines for Toxicity Rating in Healthy Individuals Participating in Vaccine Studies [17]. All AEs that are not mentioned in the guideline will be classified as mild, moderate, or severe:

- **Mild:** Any reaction, sign, or symptom that a person may find but does not interfere with a person's everyday activities and routine
- **Moderate:** Any reaction, sign, or symptom that is annoying enough to interfere with a person's normal daily activities, which may require medical intervention.
- **Severe:** Any reaction, symptom, or symptom causes a great deal of discomfort that significantly interferes with a person's daily activities and poses disability or health risk. These cases usually require medical intervention.

Relation to intervention

To make the connection between AE or Adverse Drug Reaction (ADR) and the intervention, 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 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. 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 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.
- **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.

Responsibilities and management

All undesirable and unexpected AEs after the vaccine candidate or placebo administration will be planned to be accurately recorded in the eCRF. It will be required to record and report the thorough history of the AE and the specifications of the vaccine candidate/placebo injected in the eCRF. The AE report will be in line with the guidelines of the FDA on how to report safety during clinical studies. The PI will be required to follow up all participants with AEs until they become symptom-free, and the patient's condition returns to normal.

According to national regulatory requirements, The PI will be required to immediately inform the representative of the pharmaceutical company, the National Research Ethics Committee, and the IFDA if any SAEs resulted in the death or risk of death of a participant within 24 hours. For SAEs with no chance of death, the PI will be required to inform the representative of the pharmaceutical company, the National Research Ethics Committee and the IFDA within a maximum of 48 hours.

Monitoring and Auditing

Monitoring visits will be performed in four situations: pre-initiation, initiation, routine follow-up, and study termination. During the pre-initiation monitoring visit, all executive members of the study team, the Clinical Research Associate (CRA), and the auditor reviewed and discussed the main protocol features. Following the recruitment of the first volunteer to the study, an initiation monitoring visit will be performed to supervise all steps regarding participation, obtaining informed consent, injection, data gathering and adverse event management at the study site. Routine follow-up monitoring visits will be performed daily to track the data gathering and

management at the study site. The PI and the executive team planned to hold the study termination monitoring visit to close the study site. The study site's closure and the trial's termination will be performed following a 14-day follow-up of all participants, gathering and approval of study data. It is worth mentioning that monitoring visits will be performed in every study center in six cities monthly by auditors. It is worth noting that the sponsor representative will audit all monitoring steps.

Discussion

This appendix presents the protocol of a randomized, placebo-controlled, double-blind, multicenter Phase III clinical trial to assess the efficacy and safety of an inactivated anti-SARS-CoV-2 vaccine candidate, BIV1-CovIran, with the participation of 20,000 participants in six cities of Iran.

Effective and safe vaccines against SARS-CoV-2 are needed to contain the COVID-19 pandemic. Using various vaccine production platforms, approximately 135 vaccine candidates are in clinical development globally, 41 of which are already in phase II/III clinical trials[18]. Despite the impressive achievements in vaccine production, the virus is moving more briskly than vaccine distribution[19]. Worldwide, vaccine distribution is shaped by challenging political, economic, social, diplomatic, and health-related matters. The vast majority of vaccination rollout against COVID-19 has occurred in high- and High-come countries[20]. More than 56 Countries, particularly countries located in African regions, did not meet the WHO goal of reaching 10% public rollout in every country until September 2020[20].

BIV1-CoVIran, the first homegrown vaccine candidate in Iran, is an inactivated whole-virus particle vaccine candidate manufactured by Shifa-Pharmed Industrial group. The preclinical evaluation of potency and safety of the vaccine candidate was performed in animal models of mice, rabbits, pigs, and monkeys [14]. In addition, to evaluate the safety and immunogenicity of the vaccine candidate, phase I and Phase II clinical trial studies were performed among 88 and 280 participants. All AEs were mild or moderate and transient in phase I and phase II, and no AEs of special interest were reported. The humeral immunity response in phase II participants revealed an 82.8% seroconversion rate for neutralizing antibodies 14 days after the second dose injection [13].

Inactivated vaccines have been broadly used and look promising as a safe and effective option against SARS-CoV-2[21]. Considering Iran's successful experiences in the mass-production of

vaccines of this platform[22], efforts to make domestic inactivated vaccines against SARS-CoV-2 seemed reasonable. Nevertheless, all the clinical trial phases of BIV1-CoVIran have been conducted in Iran, and the vaccine has not been studied on ethnicities beyond the Iranian population, which needs to be addressed in future studies.

Ethics and Dissemination

Research ethics approval

The Phase III clinical trial procedures related to conducting the study, record retention, data collection, and application process for the approval of IFDA will be carried out in compliance with GCP and the standard operation procedure (SOP) at Shifa-Pharmed Industrial Group. The study will abide by the declaration of Helsinki, and IR-GCP. The National Research Ethics Committee approved the study protocol under the reference code of IR.NREC.1399.008 before initiation of the study. The study protocol will be fully explained to volunteers in the screening session, and all participants will provide written informed consent before enrollment. All data will be stored on high-security servers with limited access. Laboratory samples, study reports, data collection forms and executive processes of the participants will be marked with randomization codes. In addition, all participants will be under the clinical trial health insurance. All expenditures regarding complications related to participating in the study will be paid or reimbursed to the bidder through insurance provided by the study sponsor. It is worth mentioning that after emergency approval authorization of the BIV1-CovIran vaccine candidate by IFDA, all participants who received a placebo during the clinical trial would be a priority for vaccination

Consent

The PI must fully explain the study's objectives to the study volunteer. An information form should be provided to the participants. All participants will be given enough time to decide whether to participate in the study and the confidentiality of their information for research purposes. All participants and their companions would sign the informed consent form and date. Participants will be aware of being completely free to refuse or permit to use their information and withdraw at any time for any reason. Similarly, the investigator or sponsor is allowed to exclude the participant at any time for executive reasons. Any other requirements for protecting participants' rights will be explained per the GCP guidelines and national laws for clinical trials and the Helsinki Recommendation.

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Confidentiality

Any study-related information will be kept confidential at the study site. All participants' information 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, including 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, 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 are required to sign and observe the principles of confidentiality of all study participants.

Ancillary and post-trial care

Ancillary and post-trial care will be provided to the participants for one year after participation by the principal investigator via the financial support of the sponsor. These treatments will include periodic visits to participants, and medical care regarding the occurrence of disease or vaccine candidate complications, as well as priority in receiving services is needed. Post-trial support is done through the communication of the volunteers with the representative physician of the company. Clinical trial insurance would cover all expected and unexpected study-related complications for all participants throughout the study.

Dissemination policy

After the termination of the study, an integrated clinical and statistical study report shall be written by the Sponsor. Any publication of the result of this trial must be consistent with the preliminary result. No trial results should be published prior to finalizing the Clinical Study Report (CSR). The present trial will be published in a clinical trial registry, indicating the trial dates and indication as well as the number of sites and locations. The patient's identity should be kept confidential.

Abbreviations

ADR: Adverse Drug Reaction

AE: Adverse Event

BSL: Biosafety level

CONSORT: Consolidated Standards of Reporting Trials

COVID-19: Coronavirus Disease 2019

CRA: Clinical Research Associate

CRO: Contract Research Organization

CSR: Clinical Study Report

DSMB: Data and Safety Monitoring Board

eCRF: Electronic Case Report Form

ELISA: Enzyme-Linked ImmunoSorbent Assay

GMR: Geometric Mean Ratio

GMT: Geometric Mean Titres

HIV: Human Immunodeficiency Virus

IFDA: Iran Food and Drug Administration

IgG: Immunoglobulin G

IgM: Immunoglobulin M

IL: Interleukin

IRCT: Iranian Registry of Clinical Trials

ITT: Intention to treat

MedDRA: Medical Dictionary for Regulatory Activities

PI: Principal investigator

PPS: Per protocol set

RT-PCR: Reverse-Transcriptase Polymerase Chain Reaction

SAE: Serious Adverse Event

SARS: Severe Acute Respiratory Syndrome

SARS-COV-2: Severe Acute Respiratory Syndrome- Corona virus- 2

Th: T-helper

TNF-alpha: Tumor Necrosis Factor-Alpha

WHO: World Health Organization

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Trial register

https://en.irct.ir/trial/54881: IRCT20201202049567N3 (Registration date: 13 March 2021)

Protocol version

Version 5.0; Dated: 03/09/2021

Funding

Shifa-Pharmed Industrial Group.

Roles and responsibilities

Sponsor

Shifa-Pharmed Industrial Group

Representative: Hassan Jalili

Contact information: +98 21 9109 0245

Email: hjalili@ut.ac.ir

Responsibilities

- Providing the latest draft of the study protocol,
- Obtaining any essential approvals from legal and regulatory agencies and authorities before the study begins,
- Providing standard and identical guidelines for study investigators in various study sites,
- Providing high-quality vaccine candidate vials for various study sites,
- Providing financial credit for all predetermined activities in the study protocol,
- Providing healthcare insurance for all participants who may be harmed by participating in the trial,
- Providing protocol training courses and other study-related essentials for research personnel,
- Hiring the workforce needed to conduct and monitor the trial, and
- Providing financial credit for all costs of the study.

Principal Investigator

Minoo Mohraz, MD

Responsibilities

- Carrying a detailed study out per the study protocol approved by the IFDA and other regulatory authorities,
- Forming and managing a research team,
- Organizing and managing the protocol training sessions for all research personnel,
- Monitoring the availability of a suitable place for participation in the study,
- Monitoring the availability of a suitable warehouse for research products used in clinical trials,
- Collaborating with monitors and predetermined observers throughout the study and providing access to all study documents,
- Providing individual case reports for severe events, including deaths, to the IFDA and the National Research Ethics Committee.

Availability of data and material

The data access is limited to PI. The DSMB would also have access to data in the cases of safety emergencies.

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf Payam Tabarsi, Professor of Infectious Diseases at Shahid Beheshti University of Medical Sciences, Tehran, Iran, was the principal investigator of another SARS-CoV-2 vaccine trial. All other co-authors declare no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Funding

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Appendices

Informed consent

Dear Sir / Madam,

Hereby, you are invited to participate in the trial as mentioned above. The essential information is provided in this sheet. Kindly note that the decision on participation in the trial is solely strictly up to you. You might refuse to take part in this research study. Before signing this informed consent form, make sure that you are on the same page with us, understand all the information in this form, and resolve all your questions.

Principle Investigator: Minoo Mohraz, M.D.

1- I am thoroughly aware of the purpose of the study.

   a. By signing the form, you clearly declare that you are consented to participate in a clinical trial which aimed to assess the safety, antibody production and efficacy of an inactivated whole-virus particle anti-SARS-Cov-2 vaccine candidate manufactured by the Shifa-Pharmed Industrial Group.

   b. COVID-19 has spread rapidly across the world and has become a global pandemic. The disease is an infectious disease caused by a virus, namely SARS-CoV-2, and no definitive medication is currently available for treatment. The main route of transmission of the virus is through respiratory droplets and close contact. Everyone is susceptible to infection. Older people and those with chronic underlying conditions are at higher risk of infection and poorer. Children are at risk for infection, as well; however, symptoms among children are relatively mild. Given the urgency of using the vaccine to contain the pandemic and the global shortage in vaccine supply, it is of paramount importance to develop a safe and effective homegrown vaccine to combat the COVID-19 epidemic. The inactivated vaccine used in this study was manufactured by the Shifa-Pharmed Industrial Group and has been tested in preclinical and animal studies and has shown promising potency. This vaccine has also been experimented on human volunteers in Phase 1, and Phase 2 studies, and its safety and antibody production have been demonstrated [13]. This vaccine has been approved by the Iran Food and Drug Administration and the

National Research Ethics Committee for this Phase III clinical trial. The main purpose of this study is to closely monitor the efficacy of the current vaccine among a larger number of participants in various study sites. The vaccine is originated from a particular strain of virus (SARS-CoV-2, WIV04) which is then cultured, harvested, inactivated, clarified, concentrated, secondary inactivated, purified and finally adjuvanted with aluminum hydroxide.

2- I am thoroughly clearly aware that my participation in this trial is completely voluntarily, and I am free to refuse to take part in this research study. I am totally assured that if I refused to participate in this study, I would not be deprived of routine diagnostic and therapeutic care.

3- I am thoroughly clearly aware that I am capable of leaving the trial at any stage I desire, even after signing the current informed consent, and I would not be deprived of routine diagnostic and therapeutic care by the decision.

4- My participation in this study is as follows:

   a. Following the complete description of the process by the study physician and a thoroughly comprehensively understanding of the whole trial process and the essential requirements, I would voluntarily sign this informed consent in two copies unless I hesitate to participate or do not meet the eligibility criteria. After signing the informed consent form, the physician would ask me for a detailed medical history. I would undergo a COVID-19 RT-PCR test. If I meet the inclusion criteria based on my medical history and test results, a study number would be randomly assigned to me, and I would unknowingly receive a vaccine or placebo.

   b. Neither the participants nor the investigators know the type of shots (vaccine or placebo) injected into each participant. The shots will be injected in two doses at a 28-day interval. After injection, I need to be monitored for immediate adverse events at the injection site for 30 minutes. The second dose will be injected on day 28 after the first injection unless I meet the revaccination exclusion criteria based on the investigator's decision. After the second injection, I would be followed up by the over-the-telephone calls.

5- My possible benefits of the current study:

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a. I am thoroughly aware that this study is a study to evaluate the efficacy of the vaccine, which is not guaranteed that I will be safe from the disease after receiving it at this stage.
b. I am thoroughly clearly aware that I do not pay for vaccinations, medical examinations, or other research-related procedures. I will be charged a shipping fee for all my visits to research sites or laboratories.
c. Considering the parallel nationwide vaccination program in the country, I am thoroughly aware that I could be notified by my study group regarding vaccine or placebo groups after two months of injection and would be able to leave the study and get vaccinated according to the national program.

6- The possible side effects and risks of the study intervention on me are as follows:
a. Some local reactions might occur during the inoculation process, including pain, tenderness, redness, stiffness, and itching at the injection site. General reactions such as fever, headache, fatigue, nausea and vomiting, diarrhea, cough, allergies, and muscle or joint pain are possible, as well. These symptoms subside on their own, generally. If symptoms persist or worsen, the evaluation and symptomatic treatment is performed by physicians.
b. There might be severe allergic reactions that I am aware that I will be treated on time, considering all effective treatment options.
c. All study physicians and investigators would inform my family and me about COVID-19 prevention strategies, and in cases of confirmed infection with SARS-CoV-2 I would be fully supported by resources provided by the study sponsor.
d. I am thoroughly aware that in cases of suspicion of COVID-19 infection, the investigators would contact the contracted medical centers for essential diagnostic and treatment measures. All required costs would be free for me.
e. I am thoroughly aware that participants with placebo injections were utterly susceptible to COVID-19 infection; however, neither the participants nor the investigators know who gets the vaccine or placebo.

7- I am thoroughly aware that I am entirely free to consent or resent to participate in this study, and my reluctance would not impact the routine essential medical care.

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8- I am thoroughly aware that all my study-related information will remain confidential, and the final finding will be disseminated without mentioning my identity.

9- I am thoroughly clearly aware that National Research Ethics Committee would be able to access my information to guarantee my rights throughout the study.

10- I am thoroughly clearly aware that I am not responsible for the costs of the study interventions.

11- I am thoroughly clearly aware that Parsian Insurance Company would cover all expected and unexpected study-related complications for me.

12- I am thoroughly clearly aware that I would be able to contact the National Research Ethics Committee in case of any objections regarding the study-related process throughout the trial.

Contact information for National Research Ethics Committee:

(+98)21-8145-5618

Floor 13, Block A, Ministry of Health & Medical Education Headquarters, Tehran, Iran.

13- This form of information and informed consent has been prepared in two copies, and after signing, one will be given to me, and the other copy will be given to the principal investigator.

By signing this form, I clearly declare that I have thoroughly precisely read the whole form, have comprehensively understood all information, have resolved all concerns and questions, and informedly consent to participate in Phase 3 clinical trial of an inactivated anti-SARS-Cov-2 vaccine manufactured by Shifa-Pharmed Industrial Group. Name: National ID: Signature: Date:

By signing this form, I, as principal investigator of the Phase 3 clinical trial of an inactivated anti-SARS-Cov-2 vaccine manufactured by Shifa-Pharmed Industrial Group, thoroughly consider myself responsible for fulfilling all related obligations and ensure the safety and rights of participants throughout the study.

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Name:
Signature:
Date:

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Supplementary appendix 2

Supplement to: Efficacy and Safety of an Inactivated Virus-Particle Vaccine for SARS-CoV-2, BIV1-CovIran: Findings from a Randomised, Placebo-Controlled, Double-Blind, Multicenter Phase III Clinical Trial amid the Delta Variant Peak

Table S1. Efficacy of BIV1-CovIran vaccine for various COVID-19 outcomes among seronegative participants

Outcome	Intervention	Placebo
Number of participants	9,416	4,823
Person-years	2,252.4	1,059.3
Symptomatic COVID-19 infection		
Number of incident cases	632	520
Incidence density per 1000 person-years, (95% CI)	280.6 (259.1-303.3)	490.9 (449.6-535.0)
Vaccine efficacy, % (95% CI)	42.9 (35.8-49.1)	N/A
Severe COVID-19 infection		
Number of incident cases	122	158
Incidence density per 1000 person-years, (95% CI)	54.2 (45.0-64.7)	149.2 (126.8-174.3)
Vaccine efficacy, % (95% CI)	63.7 (54.0-71.4)	N/A
Critical COVID-19 infection		
Number of incident cases	5	14
Incidence density per 1000 person-years, (95% CI)	2.2 (0.7-5.2)	13.2 (7.2-22.2)
Vaccine efficacy, % (95% CI)	82.9 (54.7-94.6)	N/A
Death due to COVID-19		
Number of incident cases	0	2
Incidence density per 1000 person-years (95% CI)	0	1.9 (0.2-6.8)
Vaccine efficacy, % (95% CI)	N/A	N/A

Table S2. Common adverse reactions after administration of BIV1-CovIran/placebo in the safety population

Adverse events		Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
		Number of events	Participants n (%)	Number of events	Participants n (%)	Number of events	Participants n (%)
Body as a whole-general disorders	Asthenia	2	2 (0.01)	1	1 (0.02)	3	3 (0.02)
	Back pain	134	128 (0.96)	75	69 (1.04)	209	197 (0.99)
	Chest pain (non-substernal)	159	143 (1.07)	78	73 (1.1)	237	216 (1.08)
	Chest pain (substernal)	2	2 (0.01)	0	0 (0)	2	2 (0.01)
	Cold sweat	28	28 (0.21)	13	13 (0.2)	41	41 (0.21)
	Face edema	3	2 (0.01)	4	5 (0.08)	7	7 (0.04)
	Fatigue	898	816 (6.12)	450	400 (6)	1348	1216 (6.08)
	Fever	1058	945 (7.09)	524	469 (7.04)	1582	1414 (7.07)
	Mouth edema	18	16 (0.12)	4	4 (0.06)	22	20 (0.1)
	Peripheral edema	1	1 (0.01)	2	2 (0.03)	3	3 (0.02)
	Pain axillary	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Pain groin	3	3 (0.02)	0	0 (0)	3	3 (0.02)
	Pelvic pain	1	1 (0.01)	5	5 (0.08)	6	6 (0.03)
	Rigors	372	352 (2.64)	185	173 (2.6)	557	525 (2.63)
	Syncope	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Temperature changed sensation	3	3 (0.02)	2	2 (0.03)	5	5 (0.03)

Adverse events		Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
		Number of events	Participants n (%)	Number of events	Participants n (%)	Number of events	Participants n (%)
Cardiovascular disorders-general	Hypertension	25	22 (0.16)	16	14 (0.21)	41	36 (0.18)
	Hypotension	31	30 (0.22)	19	18 (0.27)	50	48 (0.24)
Central & peripheral nervous system disorders	Blepharospasm	1	1 (0.01)	2	2 (0.03)	3	3 (0.02)
	Dizziness	389	347 (2.6)	160	154 (2.31)	549	501 (2.51)
	Dysaesthesia	3	2 (0.01)	0	0 (0)	3	2 (0.01)
	Dysphonia	45	43 (0.32)	15	15 (0.23)	60	58 (0.29)
	Headache	1917	1644 (12.33)	969	827 (12.41)	2886	2471 (12.36)
	Hyperaesthesia	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Myelitis	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Paraesthesia	50	42 (0.31)	32	29 (0.44)	82	71 (0.36)
	Stupor	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Tremor	4	4 (0.03)	0	0 (0)	4	4 (0.02)
Endocrine disorders	Breast pain of the male	1	1 (0.01)	0	0 (0)	1	1 (0.01)
Gastro-intestinal system disorders	Abdominal pain	251	217 (1.63)	108	96 (1.44)	359	313 (1.57)
	Anal ulcer	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Constipation	9	9 (0.07)	5	5 (0.08)	14	14 (0.07)
	Diarrhea	589	518 (3.88)	295	275 (4.13)	884	793 (3.97)

Adverse events		Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
		Number of events	Participants n (%)	Number of events	Participants n (%)	Number of events	Participants n (%)
	Dyspepsia	11	11 (0.08)	4	4 (0.06)	15	15 (0.08)
	Flatulence	9	9 (0.07)	1	1 (0.02)	10	10 (0.05)
	Gastroesophageal reflux	2	2 (0.01)	2	2 (0.03)	4	4 (0.02)
	Hiccup	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Lip ulceration	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Melaena	2	2 (0.01)	1	1 (0.02)	3	3 (0.02)
	Mouth dry	9	9 (0.07)	3	3 (0.05)	12	12 (0.06)
	Nausea	461	427 (3.2)	225	203 (3.05)	686	630 (3.15)
	Salivary gland enlargement	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Stomatitis ulcerative	14	12 (0.09)	13	11 (0.17)	27	23 (0.12)
	Tongue disorder	4	4 (0.03)	0	0 (0)	4	4 (0.02)
	Toothache	2	1 (0.01)	1	1 (0.02)	3	2 (0.01)
	Vomiting	47	44 (0.33)	15	15 (0.23)	62	59 (0.3)
Hearing and vestibular disorders	Earache	40	38 (0.28)	17	16 (0.24)	57	54 (0.27)
	Ear disorder NOS	1	1 (0.01)	4	4 (0.06)	5	5 (0.03)
	Hearing decreased	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Tinnitus	2	2 (0.01)	1	1 (0.02)	3	3 (0.02)
Heart rate and rhythm disorders	Bradycardia	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Palpitation	57	56 (0.42)	30	29 (0.44)	87	85 (0.43)

Adverse events		Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
		Number of events	Participants n (%)	Number of events	Participants n (%)	Number of events	Participants n (%)
	Tachycardia	6	6 (0.04)	5	5 (0.08)	11	11 (0.06)
Injection site reaction	Injection site bruising	36	34 (0.25)	9	9 (0.14)	45	43 (0.22)
	Injection site infection	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Injection site Anesthesia	6	6 (0.04)	3	3 (0.05)	9	9 (0.05)
	Injection site bleeding	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Injection site induration	47	44 (0.33)	16	15 (0.23)	63	59 (0.3)
	Injection site inflammation	20	20 (0.15)	11	9 (0.14)	31	29 (0.15)
	Injection site pain	6158	5068 (38.01)	2862	2363 (35.45)	9020	7431 (37.16)
	Injection site pressure sensation	97	94 (0.7)	54	52 (0.78)	151	146 (0.73)
	Injection site pruritus	46	45 (0.34)	25	24 (0.36)	71	69 (0.35)
	Injection site rash	26	26 (0.19)	8	8 (0.12)	34	34 (0.17)
	Injection site reaction	22	21 (0.16)	8	8 (0.12)	30	29 (0.15)
	Injection site swelling	37	35 (0.26)	20	20 (0.3)	57	55 (0.28)
Metabolic and nutritional disorders	Hyperglycaemia	3	3 (0.02)	1	1 (0.02)	4	4 (0.02)
	Thirst	5	5 (0.04)	7	7 (0.11)	12	12 (0.06)
	Weight decrease	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
Musculo-skeletal system disorders	Arthralgia	47	42 (0.31)	24	23 (0.35)	71	65 (0.33)
	Arthritis	1	1 (0.01)	0	0 (0)	1	1 (0.01)

Adverse events		Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
		Number of events	Participants n (%)	Number of events	Participants n (%)	Number of events	Participants n (%)
	Arthritis infective	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Arthropathy	2	2 (0.01)	4	3 (0.05)	6	5 (0.03)
	Leg pain	99	92 (0.69)	74	71 (1.07)	173	163 (0.82)
	Muscle weakness	1000	921 (6.91)	512	465 (6.98)	1512	1386 (6.93)
	Musculoskeletal pain	18	17 (0.13)	7	5 (0.08)	25	22 (0.11)
	Myalgia	1332	1203 (9.02)	685	592 (8.88)	2017	1795 (8.98)
	Pain in extremity	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Skeletal pain	307	273 (2.05)	121	110 (1.65)	428	383 (1.92)
Myo- endo- pericardial & valve disorders	Cardiac pain	2	2 (0.01)	0	0 (0)	2	2 (0.01)
	Coronary artery disorder	2	2 (0.01)	0	0 (0)	2	2 (0.01)
Platelet-bleeding & clotting disorders	Bruise	11	10 (0.07)	4	5 (0.08)	15	15 (0.08)
Psychiatric disorders	Aggressive reaction	3	3 (0.02)	1	1 (0.02)	4	4 (0.02)
	Agitation	6	6 (0.04)	4	4 (0.06)	10	10 (0.05)
	Anorexia	41	40 (0.3)	14	12 (0.18)	55	52 (0.26)
	Appetite increased	10	9 (0.07)	4	4 (0.06)	14	13 (0.07)
	Concentration impaired	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Depression	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)

Adverse events		Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
		Number of events	Participants n (%)	Number of events	Participants n (%)	Number of events	Participants n (%)
	Insomnia	7	7 (0.05)	11	10 (0.15)	18	17 (0.09)
	Sleep disorder	6	6 (0.04)	2	2 (0.03)	8	8 (0.04)
	Somnolence	162	156 (1.17)	77	75 (1.13)	239	231 (1.16)
Reproductive disorders-female	Breast pain female	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Menorrhagia	2	2 (0.01)	0	0 (0)	2	2 (0.01)
	Menstrual disorder	32	30 (0.22)	19	18 (0.27)	51	48 (0.24)
	Vaginal haemorrhage	2	2 (0.01)	2	2 (0.03)	4	4 (0.02)
	Vaginitis	1	1 (0.01)	0	0 (0)	1	1 (0.01)
Reproductive disorders-male	Prostatic disorder	1	1 (0.01)	0	0 (0)	1	1 (0.01)
Resistance mechanism disorders	Herpes simplex	18	17 (0.13)	4	4 (0.06)	22	21 (0.11)
	Herpes zoster	3	3 (0.02)	2	2 (0.03)	5	5 (0.03)
Respiratory system disorders	Coughing	407	376 (2.82)	220	198 (2.97)	627	574 (2.87)
	Dyspnea	241	226 (1.69)	115	110 (1.65)	356	336 (1.68)
	Epistaxis	11	11 (0.08)	3	3 (0.05)	14	14 (0.07)
	Laryngitis	132	125 (0.94)	79	72 (1.08)	211	197 (0.99)
	Nasal congestion	14	14 (0.1)	8	8 (0.12)	22	22 (0.11)
	Nasal dryness	1	1 (0.01)	5	4 (0.06)	6	5 (0.03)

Adverse events		Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
		Number of events	Participants n (%)	Number of events	Participants n (%)	Number of events	Participants n (%)
	Pharyngitis	735	668 (5.01)	372	331 (4.97)	1107	999 (5)
	Rhinitis	649	595 (4.46)	323	290 (4.35)	972	885 (4.43)
	Sneezing	59	56 (0.42)	31	27 (0.41)	90	83 (0.42)
	Sputum increased	55	53 (0.4)	15	13 (0.2)	70	66 (0.33)
	Throat tightness	2	2 (0.01)	3	3 (0.05)	5	5 (0.03)
Skin and appendages disorders	Acne	5	5 (0.04)	3	3 (0.05)	8	8 (0.04)
	Alopecia	15	15 (0.11)	4	4 (0.06)	19	19 (0.1)
	Erythema induratum	2	2 (0.01)	0	0 (0)	2	2 (0.01)
	Pruritus	62	56 (0.42)	27	25 (0.38)	89	81 (0.41)
	Rash	20	20 (0.15)	12	10 (0.15)	32	30 (0.15)
	Rash erythematous	18	18 (0.13)	10	9 (0.14)	28	27 (0.14)
	Skin disorder	3	3 (0.02)	0	0 (0)	3	3 (0.02)
	Skin dry	2	2 (0.01)	1	1 (0.02)	3	3 (0.02)
	Sweating increased	98	95 (0.71)	42	40 (0.6)	140	135 (0.68)
	Urticaria	28	24 (0.18)	13	13 (0.2)	41	37 (0.19)
	Skin discolouration	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
Soft tissue disorders	Axillary mass	2	2 (0.01)	0	0 (0)	2	2 (0.01)
Special senses other-disorders	Parosmia	29	29 (0.22)	17	15 (0.23)	46	44 (0.22)
	Taste perversion	7	7 (0.05)	4	4 (0.06)	11	11 (0.06)

Adverse events		Intervention (n=13,335)		Placebo (n=6,665)		Total (n=20,000)	
		Number of events	Participants n (%)	Number of events	Participants n (%)	Number of events	Participants n (%)
	Tastes loss	9	9 (0.07)	4	4 (0.06)	13	13 (0.07)
Urinary system disorders	Bladder discomfort	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Dysuria	2	2 (0.01)	1	1 (0.02)	3	3 (0.02)
	Hematuria	4	4 (0.03)	0	0 (0)	4	4 (0.02)
	Micturition frequency	2	2 (0.01)	1	1 (0.02)	3	3 (0.02)
	Oliguria	1	1 (0.01)	0	0 (0)	1	1 (0.01)
	Urinary tract infection	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
	Urine abnormal	4	4 (0.03)	0	0 (0)	4	4 (0.02)
Vascular (extracardiac) disorders	Flushing	346	324 (2.43)	167	157 (2.36)	513	481 (2.41)
	Ocular haemorrhage	3	2 (0.01)	0	0 (0)	3	2 (0.01)
	Telangiectasis	1	1 (0.01)	1	1 (0.02)	2	2 (0.01)
Vision disorders	Conjunctivitis	21	17 (0.13)	7	7 (0.11)	28	24 (0.12)
	Diplopia	4	4 (0.03)	3	3 (0.05)	7	7 (0.04)
	Eye irritation	12	12 (0.09)	3	3 (0.05)	15	15 (0.08)
	Eye pain	25	24 (0.18)	13	13 (0.2)	38	37 (0.19)
	Lacrimation abnormal	3	3 (0.02)	3	3 (0.05)	6	6 (0.03)
	Vision abnormal	16	16 (0.12)	6	6 (0.09)	22	22 (0.11)
White cell and RES disorders	Lymphadenopathy	9	9 (0.07)	8	8 (0.12)	17	17 (0.09)

Table S3. Serious adverse event after administration of BIV1-CovIran/placebo among the study population

Preferred term	Criteria for definition of SAE	Intervention group
Respiratory insufficiency(PCR+)	Hospitalization	Vaccine
Dyspnoea(PCR+)	Hospitalization	Placebo
Dyspnoea(PCR+)	Hospitalization	Placebo
Dyspnoea(PCR+)	Hospitalization	Vaccine
Vertigo, Headache (PCR+)	Hospitalization	Vaccine
Accident NOS	Hospitalization	Placebo
Respiratory insufficiency(PCR+)	Hospitalization	Vaccine
Asthenia (PCR+)	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
Dyspnoea(PCR+)	Hospitalization	Placebo
Paralysis	Hospitalization	Vaccine
Chest pain	Hospitalization	Placebo
Lymphopenia (PCR+)	Hospitalization	Placebo
Anaphylactic shock	Life threatening	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
Tachycardia	Hospitalization	Placebo
Dyspnoea(PCR+)	Hospitalization	Vaccine
Dyspnoea	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
Myocardial Infarction	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Chest pain (PCR+)	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Renal calculus	Hospitalization	Vaccine

Preferred term	Criteria for definition of SAE	Intervention group
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Appendicitis	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
Embolism pulmonary	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Renal calculus	Hospitalization	Placebo
Myocardial Infarction	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Thrombosis venous deep	Hospitalization	Placebo
Embolism pulmonary	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Embolism pulmonary	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo

Preferred term	Criteria for definition of SAE	Intervention group
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
Chest pain	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
Bradycardia(PCR+)	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
Surgical intervention	Hospitalization	Vaccine
Appendicitis	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Hypoglycaemia	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Surgical intervention	Hospitalization	Vaccine
Retinal detachment	Medically important event	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
Infection fungal	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo

Preferred term	Criteria for definition of SAE	Intervention group
COVID-19 infection	Hospitalization	Placebo
Chest pain	Medically important event	Placebo
COVID-19 infection	Hospitalization	Placebo
Stenosis vein	Medically important event	Vaccine
COVID-19 infection	Hospitalization	Placebo
Headache	Hospitalization	Placebo
Ataxia	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Surgical intervention	Medically important event	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
Surgical intervention	Medically important event	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Surgical intervention	Medically important event	Vaccine
Surgical intervention	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Chest pain	Medically important event	Vaccine
Angioedema (PCR+)	Medically important event	Vaccine
COVID-19 infection	Hospitalization	Placebo
Surgical intervention	Medically important event	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
Surgical intervention	Medically important event	Placebo
COVID-19 infection	Hospitalization	Placebo

Preferred term	Criteria for definition of SAE	Intervention group
COVID-19 infection	Hospitalization	Placebo
Surgical intervention	Medically important event	Placebo
Diabetes mellitus	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
Surgical intervention	Medically important event	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
Chest pain	Medically important event	Vaccine
Cardiac tamponade	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Chest pain	Medically important event	Vaccine
COVID-19 infection	Hospitalization	Placebo
Surgical intervention	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Placebo
Appendicitis	Hospitalization	Vaccine
Cystitis	Hospitalization	Vaccine
Abortion	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine

Preferred term	Criteria for definition of SAE	Intervention group
Pyelonephritis (PCR+)	Hospitalization	Placebo
Arthralgia	Hospitalization	Vaccine
Surgical intervention	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
Surgical intervention	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Chest Pain	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
Abortion	Medically important event	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
COVID-19 infection	Hospitalization	Vaccine
Surgical intervention	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Vaccine
COVID-19 infection	Hospitalization	Placebo
Thrombosis venous deep	Hospitalization	Vaccine

Table S4. Deaths after administration of BIV1-CovIran/placebo among study population

Age	Sex	Preferred term	Severity	Seriousness	Reason of Death	Intervention group	Efficacy population
60	M	Death	Severe	Serious	COVID-19 encephalopathy	Vaccine	Unrelated
53	M	Death	Severe	Serious	COVID-19 side effects (Bradycardia)	Placebo	Unrelated
59	M	Death	Severe	Serious	COVID-19 side effects	Vaccine	Unrelated
53	M	Death	Severe	Serious	COVID-19 side effects (Cardiac arrest)	Placebo	Yes
57	M	Death	Severe	Serious	COVID-19 side effects (Cardiac arrest)	Placebo	Yes

Table S5. Exploratory immunogenicity assessment two weeks after second injection among 400 participants of Phase III among seronegative participants

Antibody	Geometric mean titer (µg/ml) (95% CI)		Geometric mean ratio (95% CI)	Seroconversion rate* (%) (95% CI)	
	5µg	Placebo	5µg	5µg	Placebo
Neutralizing antibody					
Day 0	1.3 (1.2, 1.5)	1.3 (1.1, 1.5)	1.1 (0.9, 1.3)	–	–
Day 42	5.2 (4.3, 6.3)	0.7 (0.6, 0.9)	7.4 (5.4, 10.1)	63.1 (56.3, 69.6)	5.3 (2.0, 11.2)
Anti-receptor binding domain IgG					
Day 0	0.4 (0.3, 0.5)	0.3 (0.2, 0.5)	1.2 (0.8, 1.8)	–	–
Day 42	7.8 (7.0, 8.8)	0.4 (0.3, 0.5)	19.9 (15.2, 26.2)	68.2 (61.6, 74.3)	8.9 (4.3, 15.7)
Anti-spike glycoprotein IgG					
Day 0	0.8 (0.6, 1.2)	1.1 (0.7, 1.9)	0.7 (0.4, 1.3)	–	–
Day 42	47.2 (40.9, 54.4)	0.8 (0.5, 1.2)	60.1 (41.4, 87.3)	76.5 (70.3, 82.0)	9.7 (5.0, 16.8)

Results reported at baseline (day 0) and two weeks after the second vaccination (day 42) for 5µg and placebo groups.
*Defined as a post-vaccination IgG titer that was at least four-fold higher than the baseline titer. Geometric mean titers for neutralizing antibody are reported in µg/ml-anti-receptor binding domain IgG in RU/ml-and anti-spike glycoprotein IgG RU/ml.
**Not applicable