PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Safety and immunogenicity of a novel inactivated virus particle vaccine for SARS-CoV-2, BIV1-CovIran: findings from double-blind, randomised, placebo-controlled, phase I and II clinical trials among healthy adults
AUTHORS	Mohraz, Minoo; Salehi, Mohammadreza; Tabarsi, Payam; Abbasi-Kangevari, Mohsen; Ghamari, Seyyed-Hadi; Ghasemi, Erfan; Pouya, Maryam Amini; Rezaei, Negar; Ahmadi, Naser; Heidari, Kazem; Malekpour, Mohammad-Reza; Nasiri, Mojtaba; Amirzargar, Ali Akbar; Saeedi Moghaddam, Sahar; Larijani, Bagher; Hosseini, Hamed; Farzadfar, Farshad

VERSION 1 – REVIEW

REVIEWER	Stephen J Thomas SUNY Upstate Medical University, Microbiology & Immunology
REVIEW RETURNED	10-Nov-2021

GENERAL COMMENTS	General: Interesting data describing early clinical trial results of a killed and adjuvanted covid vaccine. Numerous areas require clarity. Placebo safety data is curious (high rates of AEs in placebo, different from other trials) and requires some discussion? Antibody and seroconversion data in placebo group speaks to suboptimal immunogenicity assays or high force of infection. If the latter was why the vaccine group not impacted by natural infection? Manuscript requires major work. Abstract: "The immunogenicity and antibody titers" - Antibody titers are a measure of immunogenicity. Consider deleting immunogenicity. "could inactivate the wild-type" - Unclear what this is describing? Sera diluted 64 fold neutralized virus? What % neutralization? "enhance the humoral immunity of all vaccine recipients" - This phrasing is unclear. The vaccine was safe and immunogenic in a small number of volunteers? Strengths "accompanied by ever-highest politically/economically induced unilateral sanctions" - The relevance of this statement is unclear without an explanation. "This study was amongst few studies" - Multiple vaccine developers have published immunogenicity data based on multiple antibody readouts. Not certain this statement is accurate.

	Introduction Methods Which staff were unblinded? Study design Setting -Confirm this was a single center study? - A statement describing which variant(s) was circulating at the time of the trial would be valuable. Patient and public Participants - Failure to enroll those at risk of infection or at risk of a bad outcome if infected is different than many trials of covid vaccines. What was the IRB rationale for this? Enrollment Procedures - What does of alhydrogel was used? - What is the placebo? Follow up "In case of suspicion for COVID-19" - What signs or symptoms were considered suspicious? Outcomes "The adverse events of special interest (AESI) defined for COVID-19 vaccines" - Reference? "Neutralising antibody titers are presented as values of the highest dilution inhibiting CPE formation" - What percent inhibition (50%)? Statistical Results "and 6/8 (75.0%) in the placebo" - Why are the rates of solicited and unsolicited AEs so high in the placebo group? "and 37.5% (8.5-75.5) in the placebo group" - Why is the placebo group experiencing seroconversion? PHASE II "After the first injection, eleven participants were excluded" - Why? "neutralising antibodies with the rate of 82.8 (77.0-87.6) versus 25.5 (14.7-39.0) in the control group" - How is the 25% seroconversion in controls explained? "effectively deactivated wild-type" - As above, not sure what this means. Discussion "there were no significant differences in safety among the study groups" - Why did placebo group have similar reactogenicity to treatment group at high levels? Adjuvant? Deserves attention.

	"participants in the placebo group have been exposed to the virus" - What was the surveillance system being employed in the study? Was it insufficiently sensitive? Discussion - The concluding statements seem to overreach the sample size and the data. Consider a more measured summary. Comparisons to inactivated covid vaccines with existing efficacy data would strengthen the paper. Thoughts on the immunogenicity results in the placebo group and why there appears to be a signal would strengthen the paper. Is this an assay issue or force of infection in the cohort? If the latter, was this seen in the vaccine group as well and how was this determined or not determined? Table 3. Geometric mean ratios of neutralising-anti-receptor-binding domain-and anti-spike glycoprotein antibodies at different time points in Phase I - Neutralizing ab responses appear not particularly robust (4 fold rise). Deserves discussion.

REVIEWER	Ana Júlia Pinto Fonseca Sieuve Afonso Universidade de São Paulo
REVIEW RETURNED	15-Nov-2021

GENERAL COMMENTS	Important papaer!

REVIEWER	Odilon Nouatin Centre de Recherches Médicales de Lambaréné
REVIEW RETURNED	10-Dec-2021

GENERAL COMMENTS	The manuscript is very well written and the results are really interesting, showing that a good job has been done. However, answers to questions, as well as some clarifications in the manuscript could be provided. - Introduction It would be nice to provide the number of cases as well as the mortality due to COVID-19 in the country in general, and in the study area particularly. - Methods * Was the vaccine safety tested in participants aged 51-75 years (Stage II, Phase I)? Why constitute a second group of the same age while this group (Stage II, Phase I) can be used to assess the immunogenicity and efficacy of the vaccine? * The different blood sampling times are not well specified. It is said in the text that visits were performed on day D28 (injection of the second dose) and day 42, but it is not specified that blood samples were taken at these different time points. A " sample collection" section would be nice. * How to justify the separation of the study population in this way (18 - 50; 51 - 75)? Is it based on a median of age? * Authors should specify the sensitivity and specificity of the Elisa kits used. - Results

	* For some graphs in the control group,, we see a reactivity following immunization compared to baseline even this is not statistically significant... Were participants in this group exposed during the vaccination period? Has a molecular test been done? * It would be very interesting to show the magnitude of response to the vaccine (ratio D84/D0, D42/D28 and D42/D0), then compare these results between vaccination groups. * The efficacy of the vaccine was evaluated via the neutralizing capacity of the antibodies. Do the authors think that this factor is sufficient enough to attest to the effectiveness of a vaccine? * It would be very interesting to quantify the B memory response, and the cytotoxic activity of CD8+ T cells. - Discussion Very well written.

REVIEWER	Alessandro Rovetta Mensana srls, Research and Disclosure Division
REVIEW RETURNED	13-Dec-2021

GENERAL COMMENTS	General comments Dear authors, thank you for the opportunity to read your interesting work. This paper summarizes the results and methods of a double-blind, randomized, placebo-controlled phase 1 and 2 for COVID-19 vaccine "2 BIV1-CovIran." In particular, the authors evaluated immunogenicity and safety. However, at present, I believe it is necessary to adequately address some critical issues to ensure transparency and reproducibility of statistical analysis. ========== Major comments 1) Introduction. It would be appropriate to specify that, in the current scenario, mRNA vaccines have greater efficacy against variants of concern (e.g., https://pubmed.ncbi.nlm.nih.gov/34579226/, https://www.nejm.org/doi/full/10.1056/nejmoa2108891). Alongside that, heterologous vaccination should also be mentioned as an effective strategy (e.g., https://pubmed.ncbi.nlm.nih.gov/34415818/). 2) Methods. 2.1. “The groups were compared with a two sample t-test [...]” The use of the paired t-test requires the verification of some assumptions (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4667138/). Please, detail how these have been verified. 2.2. “[...] mean, and standard deviation (SD) were used to describe the data.” These descriptive statistics are useful when the data is (roughly) normally distributed (https://pubmed.ncbi.nlm.nih.gov/30648682/). Please, detail how this was verified. 2.3. “The groups were compared with a two sample t-test at a two-sided 5% significance level.” P-values should be used, at best, as graded measures of the strength of evidence against the null

	hypothesis (https://pubmed.ncbi.nlm.nih.gov/28698825/, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4877414/). Therefore, I strongly suggest talking about degrees of evidence instead of fixing a threshold (e.g., P=.049 and P=.051 are similar results). For example, it is possible to speak of low, medium, and high significance. 2.4. The use of ANOVA is not mentioned in the methods. Please, add it (also specifying the type of ANOVA). Moreover, I suggest detailing how its assumptions have been verified (https://www.real-statistics.com/one-way-analysis-of-variance-anova/assumptions-anova/, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5296382/). 2.5. “Two independent samples t-test: two independent samples t-test or modified t-test is used to [...]” I suggest detailing the use of the modified t-test in the methods section. 3) Results. 3.1. I suggest rephrasing some sentences about statistical significance following my comment 2.3. Indeed, it is incorrect to claim that there is no clinical difference between two groups because P>.05. Also, I suggest distinguishing the effect size from the statistical significance. 3.2. I kindly ask the authors where the P-values of t-tests and ANOVA are reported. Thank you. 4) Discussion. 4.1. I strongly suggest specifying "At the time the study was conducted" (or similar phrases) when comparing the results with other vaccines. 4.2. I suggest rephrasing some sentences about statistical significance following my comments 2.3 and 3.1. ========== Minor comments m1) Introduction. “These vaccines have been used for emerging respiratory diseases and hold promise for a safe, effective, and inexpensive option against SARS-CoV-2 [13].” Since reference 13 does not mention COVID-19, I suggest providing a more appropriate reference. m2) Strengths and limitations of this study. I suggest separating "Strengths" and "Limitations" to get more clarity.

REVIEWER	Ana Gonçalves Universidade Federal do Rio Grande do Norte
REVIEW RETURNED	14-Dec-2021

GENERAL COMMENTS	I considered it an excellent article. The manuscript is well organized and is well written. It covers all requirements requested by the journal guidelines for clinical trials. The subject is current, interest not only to the scientific community but also to the general population. Since it directly benefits the

	health of the population involved in the study. Tables, figures, and supplementary material are organized and with clear information.

REVIEWER	Tarun Saluja International Vaccine Institute
REVIEW RETURNED	18-Dec-2021

GENERAL COMMENTS	General. English sentences used are wrong at many places, please get it reviewed by a native English speaker Abstract Result • Page 2 Lines 31-34 "The immunogenicity and antibody titers increased more among 5μg than 3μg" This is a vague statement, please re-write the sentence and clarify what do you mean by more? Statistically significant? CI overlapping? Any pre-defined criteria for dose selection? • Please describe the result for 3μg & Placebo as well, which is main aim of stage 1, i.e dose selection. • Line 42-46, please clarify the statement "The 64-times diluted sera of 92%, 77%, and 82% of vaccinated participants could inactivate the wild-type virus in Phase I-Stage I, Phase I Stage II, and Phase II clinical trials, respectively." Add the corresponding objective/endpoint in Method section. Moreover this statement should be in conclusion section. • As this is a phase 1 study with safety as primary objective, please report data for safety events in Abstract. Strengths & limitations of the study • Page 3, lines 8-14 Please note that Journal refers to the strengths and limits of the study under discussion, statement "The public rollout of a safe domestic COVID-19 vaccine could be a valuable solution, considering the catastrophic toll of COVID-19 in Iran, accompanied by ever-highest politically/economically induced unilateral sanctions." Is true in general context but can't be mentioned as study strength Page 6, settings, please explain how social distancing & COVID 19 preventive measures were implemented among participants & study staff? Page 7, Enrollment, randomisation, and interventions please define what was used as placebo? Page 15, Immunogenicity, please mention what was the confidence interval, atleast for the 1st value in the paragraph, like 1.3 (95% CI 0.9-1.7) Page 16, Discussion, it would be good to compare incidence of AEs, seroconversion and GMT levels with other COVID 19 vaccines with same & different platforms. Page 17, please discuss the limitations of the study beside

	unavailability of the research authorised kits Page 17, conclusion, please rewrite the conclusion and avoid words like ‘enormously enhance’ ‘safety and efficacy of COVID-19 hospitalisation’

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Dr. Stephen J Thomas, SUNY Upstate Medical University

General

Reviewer
Interesting data describing early clinical trial results of a killed and adjuvanted covid vaccine. Numerous areas require clarity.
Authors
The authors appreciate the encouraging comments of the reviewer.

Reviewer
Placebo safety data is curious (high rates of AEs in placebo, different from other trials) and requires some discussion?
Authors
We appreciate the concern of the reviewer. Kindly note that at the time of the trial design in December 2020 [1], vaccination against COVID-19 was a novel concept, and the vaccination was far from initiation in Iran. Thus, the investigators acted with extra caution. In Phase I, participants resided in the clinical trial site (Eram Hotel) for up to seven days after each injection for close observation. Each participant would reside in a separate room of the hotel. In this period, twice daily clinical visits by physicians and constant monitoring by study nurses were provided to assess any related adverse events. Thus, subjective adverse events could have been over reported by some particiapnts. The safety data in the manuscript is presented as the appearance of at least one related adverse event. Considering that the placebo contains diluted Alhydrogel, the occurrence of the adverse events related to Alhydrogel (including pain in the injection site, tenderness and headache) is not unexpected [2]. Moreover, in phase II, where participants would leave the study site 30 minutes after the injection, the number participants who reported at least one related adverse event 125/224 (56.3%) among the 5µg group compared to 27/56 (46.4%) among the placebo group, which were lower than Phase I.
To enhance the clarity and readability of the manuscript, we added a section in the discussion section:
The most common adverse event in both phases was injection site pain. No vaccine-related serious or life-threatening adverse events were reported. Moreover, there were no statistically significant differences in safety among the study groups. The vaccine and the placebo both contained the same aluminium hydroxide adjuvant, a common adverse effect of which could be injection site pain and tenderness [2].

Reviewer
Antibody and seroconversion data in placebo group speaks to suboptimal immunogenicity assays or high force of infection. If the latter was why the vaccine group not impacted by natural infection? Manuscript requires major work.
Authors
The authors appreciate the meticulous comment of the reviewer. The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Considering high ongoing SARS-CoV-2 circulation at the community level

during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [4]. The authors agreed with the reviewer's opinion that the antibody response among vaccinated participants might be inflated due to subclinical COVID-19 infection.
To enhance the clarity and transparency, we discussed it in the discussion section:
The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Moreover, 10% of the participants' sera in the placebo group in 64-times dilution deactivated the wild-type virus. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [5]. Future studies need to assess whether the antibody response among vaccinated participants could be inflated due to subclinical COVID-19 infection.

Abstract

Reviewer
"The immunogenicity and antibody titers"
Antibody titers are a measure of immunogenicity. Consider deleting immunogenicity.
Authors
Thank you for your fair comment. We revised the sentence, which now reads: The antibody titers increased more among 5µg than 3µg.

Reviewer
"could inactivate the wild-type"
Unclear what this is describing? Sera diluted 64 fold neutralised virus? What % neutralisation?
Authors
The authors appreciate the comment of the reviewer. The conventional virus neutralisation test (cVNT) was performed to evaluate vaccine protectivity and levels of functional antibodies raised against SARS-CoV-2. To enhance clarity, the sentence was revised to the following: In the conventional virus neutralisation test, the sera at 1/64 times dilution would neutralise SARS-CoV-2 among 92%, 77%, and 82% of vaccinated participants in Phase I-Stage I, Phase I-Stage II, and Phase II clinical trials, respectively.

Reviewer
"enhance the humoral immunity of all vaccine recipients"
This phrasing is unclear. The vaccine was safe and immunogenic in a small number of volunteers?
Authors
We realise that the sentence could be rather ambiguous. The sentence was revised and now reads: These results support further evaluation of this inactivated whole virus particle vaccine.

Strengths

Reviewer
"accompanied by ever-highest politically/economically induced unilateral sanctions"
The relevance of this statement is unclear without an explanation.
Authors
The authors appreciate the comment of the reviewer. While addressing the comments of the editor, the sentence was omitted.

Reviewer
"This study was amongst few studies"
Multiple vaccine developers have published immunogenicity data based on multiple antibody readouts. Not certain this statement is accurate.
Authors

The authors appreciate the comment of the reviewer. While addressing the comments of the editor, the sentence was omitted.

Introduction

Methods

Reviewer
Which staff were unblinded?
Authors
Thank you for your comment. The following sentence was included in the methods section: Only the contract research organisation (CRO) was unblinded at the study site.

Study design

Setting

Reviewer
Confirm this was a single center study?
Authors
Thank you for your comment. We agree with the reviewer that this needs to be acknowledged. The fact that this was a single-centre study was acknowledged in the methods section both in the abstract and the main manuscript.

Reviewer
A statement describing which variant(s) was circulating at the time of the trial would be valuable.
Authors
Thank you for your meticulous comment. The dates of the official announcement for variants of concern in Iran is now presented in Figure 1.

Patient and public

Participants

Reviewer
Failure to enroll those at risk of infection or at risk of a bad outcome if infected is different than many trials of covid vaccines. What was the IRB rationale for this?
Authors
Thank you for your precise comment. Kindly note that the manuscript is presenting findings from Phase I and Phase II studies. In Phase I, similar to the clinical trials of other vaccine candidates [6], volunteers with increased risk for severe COVID-19 were excluded. As mentioned in the study protocol (Supplementary Appendix 2), during Phase II, neurological or pulmonary severe diseases in medical examinations and according to the volunteer history (significant change in the course of treatment or hospitalisation due to exacerbation of the disease in the last three months) were excluded. However, all mild to moderate patients with the controlled disease, like other healthy individuals, were able to attend the study.
To enhance clarity and readability, the corresponding paragraph in the methods was revised, which now reads:
Participants aged 18-75 years who did not have a history of COVID-19, documented via medical history and negative serological screening, and were not infected with SARS-CoV-2 at the time of screening, documented via a negative real-time reverse transcription polymerase-chain-reaction (RT-PCR), the absence of suspicious symptoms, and no contact with a person with confirmed SARS-CoV-2 infection in the past 14 days, were included. The serological screening was performed using enzyme-linked immunosorbent assay (ELISA) kits: PT-SARS-CoV-2.IgM-96 [7] and PT-SARS-CoV-2.IgG-96 [8], Pishtaz Teb [9], Tehran, Iran.
In Phase I, volunteers with increased risk for severe COVID-19 were excluded. During Phase II, volunteers with any uncontrolled diseases like uncontrolled blood pressure (systolic and diastolic blood pressure above 140 and 90mmHg, respectively), diabetes, chronic heart, kidney, liver,

neurological or pulmonary severe diseases in medical examinations and according to the volunteer history (significant change in the course of treatment or hospitalisation due to exacerbation of the disease in the last three months) were excluded. However, all mild to moderate patients with the controlled disease, like other healthy individuals, were able to attend Phase II of the study. Other key exclusion criteria included a self-reported history of severe allergic reactions, known allergy to vaccine ingredients, genetic, congenital, or neurologic disorders, chronic renal, hepatic, or pulmonary diseases, malignancy, immunodeficiency, coagulation abnormalities, tuberculosis, and hepatitis B or C. Pregnant or breastfeeding volunteers, women who had an intention to get pregnant in the following year, and those who did not plan to use contraception during the study period were also excluded. Receiving a live attenuated vaccine in the prior month, or any vaccines in the past 14 days, as well as receiving immunosuppressive medication, immunoglobulin or blood products during the past three months, led to exclusion from the clinical trial. Notably, participants were advised to delay other live or attenuated vaccine injections up to at least one month after receiving the last dosage of the vaccine; however, exceptions were considered in case of an urgent indication for vaccination, such as for rabies post-exposure prophylaxis. Individuals with occupations that were deemed high-risk for SARS-CoV-2 exposure (e.g., healthcare professionals) did not enter the study. Further details about screening and eligibility criteria are available in the summary of study protocols [1,10,11].

Enrollment

Procedures

Reviewer

What does of alhydrogel was used?

Authors

The authors appreciate the comment of the reviewer. Alhydrogel, a vaccine adjuvant consisting of Aluminium hydroxide gel 2% (referred to as Alum), was used in the vaccine production. Each dose of vaccine included a maximum of 500 µg of Alhydrogel. Each dose of placebo included a maximum of 500 µg of Alhydrogel, which was diluted by phosphate-buffered saline.

The paragraph describing the vaccine was revised and now reads:

BIV1-CovIran is an inactivated whole virus particle vaccine manufactured by Shifa Pharmed Industrial Group. The SARS-CoV-2 virus was isolated from the nasopharyngeal specimen of an Iranian patient with COVID-19. The virus was sequenced and cultured using a Vero cell manufacturing platform in a biosafety level 3 (BSL-3) facility [12]. Viral particles were inactivated with β-propiolactone. After purification, the inactivated virus particles were sterilised with filtration and formulated with Alhydrogel as adjuvant (Croda International [13]). 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.

Reviewer

What is the placebo?

Authors

Thank you for your comment. Each dose of placebo included a maximum of 500 µg of Alhydrogel, which was diluted by phosphate-buffered saline.

Follow up

Reviewer

"In case of suspicion for COVID-19"

What signs or symptoms were considered suspicious?

Authors

The authors appreciate the precise comment of the reviewer. The following sentences were included in the follow-up section of the methods:

Suspected COVID-19 cases were defined as presenting at least two of the following symptoms: fever (axillary temperature ≥37.5 °C), chills, sore throat, stuffy nose, myalgia, fatigue, headache, nausea or vomiting, or diarrhoea; OR at least one respiratory sign or symptom (including cough, shortness of breath), new olfactory or taste disorder, radiographic evidence of COVID-19 like pneumonia.

Outcomes

Reviewer
"The adverse events of special interest (AESI) defined for COVID-19 vaccines"
Reference?
Authors
Thank you for your meticulous comment. The references was included in the sentence ,which now reads: The adverse events of special interest (AESI) defined for COVID-19 vaccines were investigated in the study [15].

Reviewer
"Neutralising antibody titers are presented as values of the highest dilution inhibiting CPE formation"
What percent inhibition (50%)?
Authors
The authors appreciate the comment of the reviewer. The Reed-Muench method was applied to calculate the neutralising antibody titre that reduced the number of infected wells by 90% [16,17]. To address this comment, this sentence was included in the methods section of the manuscript.

Statistical

Results

Reviewer
"and 6/8 (75.0%) in the placebo"
Why are the rates of solicited and unsolicited AEs so high in the placebo group?
Authors
The authors appreciate the concern of the reviewer. Kindly note that at the time of the trial design in December 2020 [1], vaccination against COVID-19 was a novel concept, and the vaccination was far from initiation in Iran. Thus, the investigators acted with extra caution. In Phase I, participants resided in the clinical trial site (Eram Hotel) for up to seven days after each injection for close observation. Each participant would reside in a separate room of the hotel. In this period, twice daily clinical visits by physicians and constant monitoring by study nurses were provided to assess any related adverse events. Thus, subjective adverse events could have been over reported by some particiapnts. The safety data in the manuscript is presented as the appearance of at least one related adverse event. Considering that the placebo contains diluted Alhydrogel, the occurrence of the adverse events related to Alhydrogel (including pain in the injection site, tenderness and headache) is not unexpected [2]. Moreover, in phase II, where participants would leave the study site 30 minutes after the injection, the number participants who reported at least one related adverse event 125/224 (56.3%) among the 5µg group compared to 27/56 (46.4%) among the placebo group, which were lower than Phase I.
To enhance the clarity and readability of the manuscript, we added a section in the discussion section:
The most common adverse event in both phases was injection site pain. No vaccine-related serious or life-threatening adverse events were reported. Moreover, there were no statistically significant differences in safety among the study groups. The vaccine and the placebo both contained the same aluminium hydroxide adjuvant, a common adverse effect of which could be injection site pain and tenderness [2].

Reviewer
"and 37.5% (8.5-75.5) in the placebo group"
Why is the placebo group experiencing seroconversion?

Authors
The authors appreciate the precise concern of the reviewer. The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [4]. The authors agreed with the reviewer's opinion that the antibody response among vaccinated participants might be inflated due to subclinical COVID-19 infection.
To enhance the clarity and transparency, we discussed it in the discussion section:
The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Moreover, 10% of the participants' sera in the placebo group in 64-times dilution deactivated the wild-type virus. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [5]. Future studies need to assess whether the antibody response among vaccinated participants could be inflated due to subclinical COVID-19 infection.

PHASE II

Reviewer
"After the first injection, eleven participants were excluded"
Why?
Authors
The authors appreciate the precise concern of the reviewer. In Phase II, as presented in Figure 4, eleven participants were excluded after receiving the first vaccine dose. The reasons for exclusion included positive SARS-CoV-2 RT-PCR (n=9), death due to suicide via cyanide toxicity (n=1), co-administration of another COVID-19 vaccine platform (n=1). Kindly note that while the latter participants were not eligible for immunogenicity assessment, they were included in the safety population of the study.

Reviewer
"neutralising antibodies with the rate of 82.8 (77.0-87.6) versus 25.5 (14.7-39.0) in the control group"
How is the 25% seroconversion in controls explained?
Authors
The authors appreciate the concern of the reviewer. The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [4]. The authors agreed with the reviewer's opinion that the antibody response among vaccinated participants might be inflated due to subclinical COVID-19 infection.
To enhance the clarity and transparency, we discussed it in the discussion section:
The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Moreover, 10% of the participants' sera in the placebo group in 64-times dilution deactivated the wild-type virus. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [5]. Future studies need to assess whether the antibody response among vaccinated participants could be inflated due to subclinical COVID-19 infection.

Reviewer
"effectively deactivated wild-type"
As above, not sure what this means.
Authors
The authors appreciate the precise concern of the reviewer. The conventional virus neutralisation test (cVNT) was performed to evaluate vaccine protectivity and levels of functional antibodies raised against SARS-CoV-2. We went through the manuscript and revised all sentences, which included "effectively deactivated wild-type" to enhance clarity. The sentences now read:
In cVNT, the sera at 1/64 times dilution of some 92.0% of vaccinated participants with 5µg BIV1-CovIran neutralised SARS-CoV-2. In contrast, zero per cent of the participants' sera at the same dilution neutralised the virus in the placebo group (Figure 6).
In cVNT, the sera at 1/64 times dilution of some 77.0% of vaccinated participants with 5µg BIV1-CovIran neutralised SARS-CoV-2. In contrast, one-fourth of the participants' sera at the same dilution neutralised the virus in the placebo group (Figure 6).
In cVNT, the sera at 1/64 times dilution of some 82.0% of vaccinated participants with 5µg BIV1-CovIran neutralised SARS-CoV-2 on day 42. In contrast, less than 10% of the participants' sera at the same dilution neutralised the virus in the placebo group (Figure 6).

Discussion

Reviewer
"there were no significant differences in safety among the study groups"
Why did placebo group have similar reactogenicity to treatment group at high levels? Adjuvant? Deserves attention.
Authors
The authors appreciate the concern of the reviewer. Kindly note that at the the safety data in the manuscript is presented as the appearance of at least one adverse event. Considering that the placebo contains diluted Alhydrogel, the occurrence of the adverse events related to Alhydrogel (including pain in the injection site, tenderness and headache) is not unexpected [2]. As for the reactogenicity witnessed in the placebo group, please note that the clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [4]. The authors agreed with the reviewer's opinion that the antibody response among vaccinated participants might be inflated due to subclinical COVID-19 infection.
To enhance the clarity and readability of the manuscript, we added a section in the discussion section:

1. The most common adverse event in both phases was injection site pain. No vaccine-related serious or life-threatening adverse events were reported. Moreover, there were no significant differences in safety among the study groups. The vaccine and the placebo both contained the same aluminium hydroxide adjuvant, a common adverse effect of which could be injection site pain and tenderness [2].
2. The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Moreover, 10% of the participants' sera in the placebo group in 64-times dilution deactivated the wild-type virus. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the

virus. This could result in seroconversion among the placebo group, reported earlier as well [5]. Future studies need to assess whether the antibody response among vaccinated participants could be inflated due to subclinical COVID-19 infection.

Reviewer

"participants in the placebo group have been exposed to the virus"

What was the surveillance system being employed in the study? Was it insufficiently sensitive?

Authors

The authors appreciate the comment of the reviewer. Kindly note that in Phase I, participants resided in the clinical trial site (Eram Hotel) for up to seven days after each injection for close observation. Each participant would reside in a separate room of the hotel. Mask use was obligatory in the shared spaces. In this period, twice daily clinical visits by physicians and constant monitoring by study nurses were provided to assess any related adverse events (AEs). Upon home discharge, the preventive measures instructions were provided. Participants were instructed to record their symptoms at home and fill out diary cards designed for this purpose. Moreover, follow-up phone calls by study nurses were made on a daily basis. In Phase II, the procedures remained the same, while participants would not reside in the hotel.

Reviewer

The concluding statements seem to overreach the sample size and the data. Consider a more measured summary. Comparisons to inactivated covid vaccines with existing efficacy data would strengthen the paper. Thoughts on the immunogenicity results in the placebo group and why there appears to be a signal would strengthen the paper. Is this an assay issue or force of infection in the cohort? If the latter, was this seen in the vaccine group as well and how was this determined or not determined?

Authors

Thank you for your comment. While addressing the previous comments of the reviewer, the conclusion section of the manuscript was revised and now reads:

Administration of the two shots of 5µg dose BIV1-CovIran vaccine with a 28-day interval would enhance the immunity of all vaccine recipients against SARS-CoV-2 with no vaccine-related SAEs. These results support further evaluation of this inactivated whole virus particle vaccine in Phase III. Furthermore, the discussion also underwent major revisions. We hope that these modifications address the concerns and comments of the reviewer.

Tables

Reviewer

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

Neutralising ab responses appear not particularly robust (4 fold rise). Deserves discussion.

Authors

The authors appreciate the comment of the reviewer. Table 3 presents the geometric mean ratios (GMRs) of neutralising-anti-receptor-binding domain-and anti-spike glycoprotein antibodies at different time points in Phase I, which was defined as the ratio of geometric mean titers (GMTs) in the vaccine group to the corresponding titers in the placebo group at the same time point (GMTs are presented in Table 2). Concurrently, we also assessed the proportion of participants who had a four-fold increase in the antibody titers at different time points within the vaccine/placebo groups in Table 4. The results showed that the seroconversion rate (95% CI) of neutralising antibodies 14 days after the second dose of vaccine injection was 45.8% (25.6–67.2) in the 3µg group, 70.8% (48.9-87.4) in the 5µg group, and 37.5% (8.5-75.5) in the placebo group in Phase I-Stage I (participants aged 18-50 years). In Phase I-Stage II (participants aged 51-75), the seroconversion rates of neutralising antibody at day 28 from the first injection in the 5µg group were 100.0 (84.6-100.0). In Phase I, the immune response induced by the 5µg dosage among participants aged 18-50 years was more prominent and

persistent than the 3μg dosage. Thus, the 5μg dosage was selected for Stage II of Phase I and Phase II clinical trials.

To enhance clarity of the manuscript, the methods section was reviewed to make sure that the definitions for geometric mean ratio and seroconversion rate were included.

Reviewer 2

Dr. Ana Júlia Pinto Fonseca Sieuve Afonso, Universidade de São Paulo

Reviewer
Important paper!
Authors
The authors appreciate the encouraging comments of the reviewer.

Reviewer: 3

Dr. Odilon Nouatin, Centre de Recherches Médicales de Lambaréné

Reviewer
The manuscript is very well written, and the results are really interesting, showing that a good job has been done. However, answers to questions, as well as some clarifications in the manuscript could be provided.
Authors
The authors appreciate the encouraging comments of the reviewer.

Introduction

Reviewer
It would be nice to provide the number of cases as well as the mortality due to COVID-19 in the country in general, and in the study area particularly.
Authors
The authors appreciate the comment of the reviewer. The following sentence was included in the introduction:
COVID-19 has resulted in more than 4 million reported cases and 93 thousand confirmed deaths in Iran, as of 6 August 2021 [3].

Methods

Reviewer
Was the vaccine safety tested in participants aged 51-75 years (Stage II, Phase I)? Why constitute a second group of the same age while this group (Stage II, Phase I) can be used to assess the immunogenicity and efficacy of the vaccine?
Authors
Thank you for your comment. All participants of the study were included in the safety population. Kindly note that at the time of the trial design in December 2020 [1], vaccination against COVID-19 was a novel concept, and the vaccination was far from initiation in Iran. Thus, the ethical committee acted with extra caution and did not allow a phase I clinical trial to be conducted among people aged>50 without evidence of safety among younger age groups. Thus, Phase I clinical trial was conducted in two stages, with the first stage focusing on people aged 18-50. Once the preliminary evidence for the vaccine's safety was provided for the ethical committee, permission for the conduction of Stage II among individuals aged 51-75 was granted. Moreover, participants aged 51-75 years were not recruited in Phase II, until safety results from that age group in Phase I were available. The previous part was also discussed in the discussion section of the manuscript.

Reviewer
The different blood sampling times are not well specified. It is said in the text that visits were performed on day D28 (injection of the second dose) and day 42, but it is not specified that blood samples were taken at these different time points. A " sample collection" section would be nice.
Authors
The authors appreciate the comment of the reviewer. The detailed schedule of the trial was included in the study protocol (Supplementary Appendix 2). To address the reviewer's comment, while also considering the Journal's word limit, the following sentences were added to their corresponding sections in methods:
Phase I
Blood samples were collected on days 7, 14, 21, and 28 after the first injection.
Phase II
Blood samples were collected on days 28 and 42 after the first injection.

Reviewer

How to justify the separation of the study population in this way (18 - 50; 51 - 75)? Is it based on a median of age?

Authors

Thank you for your comment. All participants of the study were included in the safety population. Kindly note that at the time of the trial design in December 2020 [1], vaccination against COVID-19 was a novel concept, and the vaccination was far from initiation in Iran. Thus, the ethical committee acted with extra caution and did not allow a phase I clinical trial to be conducted among people aged>50 without evidence of safety among younger age groups. Thus, Phase I clinical trial was conducted in two stages, with the first stage focusing on people aged 18-50. Once the preliminary evidence for the vaccine's safety was provided for the ethical committee, permission for the conduction of Stage II was granted. Moreover, participants aged 51-75 years were not recruited in Phase II, until safety results from that age group in Phase I were available.

The previous part was also discussed in the discussion section of the manuscript.

Reviewer

Authors should specify the sensitivity and specificity of the Elisa kits used.

Authors

Thank you for your comment. The corresponding sentence in the methods section was revised, which now reads: PT-SARS-CoV-2.IgM-96 (the reported sensitivity and specificity: 79.4% and 97.30%, respectively) [7] and PT-SARS-CoV-2.IgG-96 (the reported sensitivity and specificity: 91.1% and 98.3%, respectively) [8], Pishtaz Teb [9], Tehran, Iran.

Results

Reviewer

For some graphs in the control group, we see a reactivity following immunisation compared to baseline even this is not statistically significant... Were participants in this group exposed during the vaccination period? Has a molecular test been done?

Authors

The authors appreciate the concern of the reviewer. The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [4]. The authors agreed with the reviewer's opinion that the antibody response among vaccinated participants might be inflated due to subclinical COVID-19 infection.

To enhance the clarity and transparency, we discussed it in the discussion section:

The clinical trial phases were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [3]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Moreover, 10% of the participants' sera in the placebo group in 64-times dilution deactivated the wild-type virus. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants in the placebo group have been exposed to the virus. This could result in seroconversion among the placebo group, reported earlier as well [5]. Future studies need to assess whether the antibody response among vaccinated participants could be inflated due to subclinical COVID-19 infection.

Reviewer

It would be very interesting to show the magnitude of response to the vaccine (ratio D84/D0, D42/D28 and D42/D0), then compare these results between vaccination groups.

Authors

The authors appreciate the meticulous comment of the reviewer. The comparison of antibody responses within various groups in both phases was included in Figure 5. We hope that this figure

provides further insights to the immunogenicity of vaccine in the study. Nevertheless, we would be happy to make further modifacations, if deemed necessary.

Reviewer
The efficacy of the vaccine was evaluated via the neutralising capacity of the antibodies. Do the authors think that this factor is sufficient enough to attest to the effectiveness of a vaccine?
Authors
Thank you for your comment. Evaluation of the neutralising capacity of the antibodies induced by vaccines, a reflection of immunogenicity, is a renowned and well-established proxy for vaccine efficacy, which has also been investigated in other COVID-19 phase I/II studies vaccines [18,19]. We agree with the reviewer's opinion that further vaccine evaluations are required. At the end of the manuscript, it was concluded that the results of phase I/II trials support further evaluation of this inactivated whole virus particle vaccine. Investigation of the vaccine efficacy is within the scope of phase III clinical trials, the results of which will be published separately. In the meantime, the effectiveness of the vaccine will be investigated in the future studies.

Reviewer
It would be very interesting to quantify the B memory response, and the cytotoxic activity of CD8+ T cells.
Authors
The authors appreciate the meticulous comment of the reviewer. While the assessment of cellular immunity was an objective presented in the study protocol, during the trial, the essential requirements of cellular immunity assessment were not available in the country. Thus, such results could not be presented in this study. While not all reports on phase I/II clinical trials of vaccines for SARS-CoV-2 presented results on cellular immunity [6,20], after all the necessary equipment was provided, another study was later initiated which will address such questions and concerns. As the study is still ongoing, the results will be presented in future manuscripts. The section now reads:

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

Discussion

Reviewer
Very well written.
Authors
The authors appreciate the encouraging comment of the reviewer.

Reviewer: 4

Dr. Alessandro Rovetta, Mensana srls

General comments

Reviewer

Dear authors, thank you for the opportunity to read your interesting work. This paper summarises the results and methods of a double-blind, randomised, placebo-controlled phase 1 and 2 for COVID-19 vaccine "2 BIV1-CovIran." In particular, the authors evaluated immunogenicity and safety. However, at present, I believe it is necessary to adequately address some critical issues to ensure transparency and reproducibility of statistical analysis.

Authors

The authors appreciate the encouraging comments of the reviewer.

Introduction

Reviewer

It would be appropriate to specify that, in the current scenario, mRNA vaccines have greater efficacy against variants of concern (e.g., https://pubmed.ncbi.nlm.nih.gov/34579226/, https://www.nejm.org/doi/full/10.1056/nejmoa2108891). Alongside that, heterologous vaccination should also be mentioned as an effective strategy (e.g., https://pubmed.ncbi.nlm.nih.gov/34415818/).

Authors

The authors appreciate the reviewer's concern. It is encouraging to compare the efficacy of vaccines of different platforms in the manuscript. Nevertheless, data from phase III studies are required for such comparison, and it could not be included in the introduction storyline of a phase I/II study. The safety and immunogenicity of various vaccine platforms are discussed in the discussion section of the manuscript. Please rest assured that we will compare the efficacy of BIV1-CovIran with other vaccines in the manuscript reporting results of phase III clinical trial. Kindly note that at the time of narration of this manuscript in June-August 2021, heterologous vaccination was not widely discussed among the scientific community. Moreover, the study presents the results of Phase I and II randomised placebo-controlled clinical trials of the BIV1-CovIran vaccine to assess its safety and immunogenicity. From where we are standing, discussing the effectiveness of heterologous vaccination is beyond the objectives of this study and could be addressed in future studies.

Methods

Reviewer

"The groups were compared with a two sample t-test [...]" The use of the paired t-test requires the verification of some assumptions (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4667138/). Please, detail how these have been verified.

"[...] mean, and standard deviation (SD) were used to describe the data." These descriptive statistics are useful when the data is (roughly) normally distributed (https://pubmed.ncbi.nlm.nih.gov/30648682/). Please, detail how this was verified.

"The groups were compared with a two sample t-test at a two-sided 5% significance level." P-values should be used, at best, as graded measures of the strength of evidence against the null hypothesis (https://pubmed.ncbi.nlm.nih.gov/28698825/, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4877414/). Therefore, I strongly suggest talking about degrees of evidence instead of fixing a threshold (e.g., P=.049 and P=.051 are similar results). For example, it is possible to speak of low, medium, and high significance.

The use of ANOVA is not mentioned in the methods. Please, add it (also specifying the type of ANOVA). Moreover, I suggest detailing how its assumptions have been verified (https://www.real-statistics.com/one-way-analysis-of-variance-anova/assumptions-anova/, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5296382/).

"Two independent samples t-test: two independent samples t-test or modified t-test is used to [...]" I suggest detailing the use of the modified t-test in the methods section.

I suggest rephrasing some sentences about statistical significance following my comment 2.3. Indeed, it is incorrect to claim that there is no clinical difference between two groups because P>.05. Also, I suggest distinguishing the effect size from the statistical significance.

Authors

The authors appreciate the reviewer's comment. In this study, due to the small sample size in some study groups in Phase I, we used D'Agostino's K-squared test to check the normality of the distribution. In cases of normal distribution of data, two sample t-test was used for comparing means among two groups. Otherwise, the means were compared using Mann-Whitney test. Tables 2, 6, S5, and S7-9 present Geometric Means and their corresponding 95% confidence intervals, using epitools statistical package in R programming language. Geometric Mean Ratios were used to evaluate the antibody response between the vaccine and the placebo groups, and thus the clinical significance was evaluated based on Geometric Mean Ratios. P-values were presented for statistical confirmation, as presented in Figure 6, with a significance level of 0.05. As we did not compare the means of more than two groups in one single test, the ANOVA test was not used in the statistical analysis. While using the two-sample t-test, if the variance of the two groups was not equal, the Welch correction (Welch's t-test) was used.

Results

Reviewer

I kindly ask the authors where the P-values of t-tests and ANOVA are reported. Thank you.

Authors

The authors appreciate the comment of the reviewer. The values are presented in Figure 5. We would be happy to make further modifacations, if deemed necessary.

Discussion

Reviewer

I strongly suggest specifying "At the time the study was conducted" (or similar phrases) when comparing the results with other vaccines.

Authors

Thank you for your comment. Amended.

Reviewer

I suggest rephrasing some sentences about statistical significance following my comments 2.3 and 3.1.

Authors

Thank you for your comment. Amended.

Minor comments

Reviewer

m1) Introduction. "These vaccines have been used for emerging respiratory diseases and hold promise for a safe, effective, and inexpensive option against SARS-CoV-2 [13]." Since reference 13 does not mention COVID-19, I suggest providing a more appropriate reference.

Authors

Thank you for your comment. Amended.

Reviewer

m2) Strengths and limitations of this study. I suggest separating "Strengths" and "Limitations" to get more clarity.

Authors

Thank you for your comment. We revised the Strengths and Limitation section according to the journal policy. The section now reads:

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

Reviewer 5

Dr. Ana Gonçalves, Universidade Federal do Rio Grande do Norte

Reviewer
I considered it an excellent article. The manuscript is well organised and is well written. It covers all requirements requested by the journal guidelines for clinical trials. The subject is current, interest not only to the scientific community but also to the general population. Since it directly benefits the health of the population involved in the study. Tables, figures, and supplementary material are organised and with clear information.

Authors
The authors appreciate the encouraging comments of the reviewer.

Reviewer: 6

Dr. Tarun Saluja, International Vaccine Institute

General

Reviewer
English sentences used are wrong at many places, please get it reviewed by a native English speaker
Authors
The authors appreciate the reviewer's opinion. We asked a bilingual native English professor at our institution to thoroughly review and revise the manuscript regarding possible grammar and syntax errors to ensure enhanced readability. In addition, we used "Grammarly", a cross-platform cloud-based writing assistant that reviews spelling, grammar, punctuation, clarity, engagement, and delivery mistakes.

Abstract

Reviewer
Page 2 Lines 31-34: "The immunogenicity and antibody titers increased more among 5µg than 3µg" This is a vague statement, please re-write the sentence and clarify what do you mean by more? Statistically significant? CI overlapping? Any pre-defined criteria for dose selection?
Authors
Thank you for your fair and constructive comments. The seroconversion rate of various antibodies among 5µg group was higher than the corresponding rates among 3µg group (P<0.01). After providing the preliminary results of the Stage I-Phase I clinical trial to the national regulator, they chose the 5µg dose to proceed. We went through the results section of the abstract and revised it, which now reads:
All adverse events (AEs) were mild or moderate and transient in both Phase I and Phase II, and no AEs of special interest were reported. The seroconversion-rate of neutralising, anti-receptor binding-domain (RBD), and anti-spike-glycoprotein (anti-S) antibodies 14-days after second dose of 5µg-vaccine in Stage I was 70.8% (95% CI 48.9-87.4), 87.5% (67.6-97.3), 91.7% (73.0-99.0). The antibody titers increased more among 5µg than 3µg. The corresponding rates for 3µg vaccine were 45.83 (25.55-67.18), 54.17 (32.82-74.45), and 70.83 (48.91-87.38), respectively. In Stage II, 100.0% (84.6-100.0), 86.4% (65.1-97.1) and 86.4% (65.1-97.1) of participants seroconverted for neutralising, anti-RBD, and anti-S antibodies.

Reviewer
Please describe the result for 3µg & Placebo as well, which is main aim of stage 1, i.e dose selection.
Authors
Thank you for your comments. We revised the section based on the comment, which now reads:
All adverse events (AEs) were mild or moderate and transient in both Phase I and Phase II, and no AEs of special interest were reported. The seroconversion-rate of neutralising, anti-receptor binding-domain (RBD), and anti-spike-glycoprotein (anti-S) antibodies 14-days after second dose of 5µg-vaccine in Stage I was 70.8% (95% CI 48.9-87.4), 87.5% (67.6-97.3), 91.7% (73.0-99.0). The antibody titers increased more among 5µg than 3µg. The corresponding rates for 3µg vaccine were 45.83 (25.55-67.18), 54.17 (32.82-74.45), and 70.83 (48.91-87.38), respectively. In Stage II, 100.0% (84.6-100.0), 86.4% (65.1-97.1) and 86.4% (65.1-97.1) of participants seroconverted for neutralising, anti-RBD, and anti-S antibodies. In Phase II, the seroconversion rate of neutralizing-antibody was 82.8% (77.0-87.6), anti-RBD 77.0% (70.7-82.6), and anti-S 79.9% (73.8-85.1) on day 42. In the conventional virus neutralisation test, the sera at 1/64 times dilution would neutralise SARS-CoV-2 among 92%, 77%, and 82% of vaccinated participants in Phase I-Stage I, Phase I-Stage II, and Phase II clinical trials, respectively.

Reviewer

Line 42-46, please clarify the statement: "The 64-times diluted sera of 92%, 77%, and 82% of vaccinated participants could inactivate the wild-type virus in Phase I-Stage I, Phase I Stage II, and Phase II clinical trials, respectively." Add the corresponding objective/endpoint in Method section. Moreover, this statement should be in conclusion section.

Authors

Thank you for your fair and constructive comments. The conventional virus neutralisation test (cVNT) was performed to evaluate vaccine protectivity and levels of functional antibodies raised against SARS-CoV-2. To enhance clarity, the sentence was revised to the following: In the conventional virus neutralisation test, the sera at 1/64 times dilution would neutralise SARS-CoV-2 among 92%, 77%, and 82% of vaccinated participants in Phase I-Stage I, Phase I-Stage II, and Phase II clinical trials, respectively.

In addition, we added the corresponding section in the primary and secondary outcome measures of the abstract. The section, now reads: Safety assessment and immunogenicity assessment via antibody response and conventional virus neutralisation test (c-VNT).

Reviewer

As this is a phase 1 study with safety as primary objective, please report data for safety events in Abstract.

Authors

Thank you for your comment. Kindly note that the manuscript provides data on both Phase I and Phase II studies. While considering the word limit of the abstract section according to the journal policy, we revised the results section of the abstract. We hope that this figure provides adequate information regarding vaccine safety and immunogenicity in both phases. The section now reads: All adverse events (AEs) were mild or moderate and transient in both Phase I and Phase II, and no AEs of special interest were reported. The seroconversion-rate of neutralising, anti-receptor binding-domain (RBD), and anti-spike-glycoprotein (anti-S) antibodies 14-days after second dose of 5µg-vaccine in Stage I was 70.8% (95% CI 48.9-87.4), 87.5% (67.6-97.3), 91.7% (73.0-99.0). The antibody titers increased more among 5µg than 3µg. The corresponding rates for 3µg vaccine were 45.83 (25.55-67.18), 54.17 (32.82-74.45), and 70.83 (48.91-87.38), respectively. In Stage II, 100.0% (84.6-100.0), 86.4% (65.1-97.1) and 86.4% (65.1-97.1) of participants seroconverted for neutralising, anti-RBD, and anti-S antibodies. In Phase II, the seroconversion rate of neutralizing-antibody was 82.8% (77.0-87.6), anti-RBD 77.0% (70.7-82.6), and anti-S 79.9% (73.8-85.1) on day 42. In the c-VNT, the sera at 1/64 times dilution would neutralise SARS-CoV-2 among 92%, 77%, and 82% of vaccinated participants in Phase I-Stage I, Phase I-Stage II, and Phase II clinical trials, respectively.

Reviewer

Page 3, lines 8-14: Please note that Journal refers to the strengths and limits of the study under discussion, statement "The public rollout of a safe domestic COVID-19 vaccine could be a valuable solution, considering the catastrophic toll of COVID-19 in Iran, accompanied by ever-highest politically/economically induced unilateral sanctions." Is true in general context but can't be mentioned as study strength.

Authors

The authors appreciate the comment of the reviewer. While addressing the comments of the editor, the sentence was omitted.

Reviewer

Page 6, settings, please explain how social distancing & COVID 19 preventive measures were implemented among participants & study staff?

Authors

The authors appreciate the comment of the reviewer. In Phase I, participants resided in the clinical trial site (Eram Hotel) for up to seven days after each injection for close observation. Each participant would reside in a separate room of the hotel. Mask use was obligatory in the shared spaces. In this period, twice daily clinical visits by physicians and constant monitoring by study nurses were provided

to assess any adverse events (AEs). Upon home discharge, the preventive measures instructions were provided. Participants were instructed to record their symptoms at home and fill out diary cards designed for this purpose. Moreover, follow-up phone calls by study nurses were made on a daily basis.
We hope that the description addresses the reviewer's comment. Nevertheless, we would be happy to make further modifacations, if deemed necessary.

Reviewer

Page 7, Enrollment, randomisation, and interventions please define what was used as placebo?

Authors

The authors appreciate the comment of the reviewer. Alhydrogel, a vaccine adjuvant consisting of Aluminium hydroxide gel 2% (referred to as Alum), was used in the vaccine production. Each dose of vaccine included a maximum of 500 µg of Alhydrogel. Each dose of placebo included a maximum of 500 µg of Alhydrogel, which was diluted by phosphate-buffered saline.
The paragraph describing the placebo was revised and now reads:
BIV1-CovIran is an inactivated whole virus particle vaccine manufactured by Shifa Pharmed Industrial Group. The SARS-CoV-2 virus was isolated from the nasopharyngeal specimen of an Iranian patient with COVID-19. The virus was sequenced and cultured using a Vero cell manufacturing platform in a biosafety level 3 (BSL-3) facility [12]. Viral particles were inactivated with β-propiolactone. After purification, the inactivated virus particles were sterilised with filtration and formulated with Alhydrogel as adjuvant (Croda International [13]). Each dose of vaccine included a maximum of 500 µg of Alhydrogel. Further details about the vaccine production are presented elsewhere [14]. The placebo solution contained the same amount of Alhydrogel, which was diluted by phosphate-buffered saline. Vaccine and placebo vials were stored at 2-8°C.

Reviewer

Page 15, Immunogenicity, please mention what was the confidence interval, at least for the 1st value in the paragraph, like 1.3 (95% CI 0.9-1.7)

Authors

Thanks for the comment. We went through the manuscript and provided the whole form of 95% confidence interval in the first appearance. The sentence now reads:
Among participants aged 18-50 years, the seroconversion rate with 95% confidence intervals (95% CI) of neutralising antibodies 14 days after the second dose of vaccine injection was 45.8% (25.6–67.2) in the 3µg group, 70.8% (48.9-87.4) in the 5µg group, and 37.5% (8.5-75.5) in the placebo group.

Reviewer

Page 16, Discussion, it would be good to compare incidence of AEs, seroconversion and GMT levels with other COVID 19 vaccines with same & different platforms.

Authors

The authors appreciate the comment of the reviewer. The comparisons were included in the discussion. The section reads:
BIV1-CovIran induced the production of neutralising antibodies, and the seroconversion rates of vaccine recipients ranged from 70.8% to 100% in Phase I and Phase II. The seroconversion rates were comparable to reports from phase I and phase II clinical trials of other SARS-CoV-2 vaccines: BBV152 [26], BBIBP-CorV [24], mRNA-1273 [46], and Ad26 and rAd5 [49].

Reviewer

Page 17, please discuss the limitations of the study beside unavailability of the research authorised kits.

Authors

Thanks for the comment. We provided the limitations of the study according to the Editor's comments. The limitations discussed in the discussion section included the following:

The ethical committee did not allow a phase I clinical trial to be conducted among people aged>50 without evidence of safety among younger age groups. Thus, Phase I clinical trial was conducted in two stages, with the first stage focusing on people aged 18-50. Once the preliminary evidence for the vaccine's safety was provided for the ethical committee, permission for the conduction of Stage II was granted. Moreover, participants aged 51-75 years were not recruited in Phase II, until safety results from that age group in Phase I were available.

The most common adverse event in both phases was injection site pain. No vaccine-related serious or life-threatening adverse events were reported. Moreover, there were no statistically significant differences in safety among the study groups. The vaccine and the placebo both contained the same aluminium hydroxide adjuvant, a commen adverse effect of which could be injection site pain and tenderness [43].

Both phases of the clinical trial were conducted when the number of daily diagnosed cases with COVID-19 was rapidly increasing [9]. In Phase II, the seroconversion of the placebo group was witnessed in 12.7% of participants on day 28 and 25.5% on day 42. Moreover, 10% of the participants' sera in 64-times dilution deactivated the wild-type virus in the placebo group. Considering high ongoing SARS-CoV-2 circulation at the community level during the clinical trial, it could be possible that participants have been exposed to the virus, which could result in seroconversion, reported earlier as well [44]. Future studies need to assess whether the antibody response among vaccinated participants could be inflated due to subclinical COVID-19 infection.
In this study, the antibody response was assessed via determining the geometric mean titres and the seroconversion rates of neutralising, anti-receptor binding-domain, and anti-spike-glycoprotein antibodies in both phases. Moreover, conventional virus neutralisation test was performed to evaluate the levels of functional antibodies raised against SARS-CoV-2 in Phase I. Nevertheless, cellular immunity induced by vaccination was not assessed in the study. The pharmaceutical company has also submitted the clinical trial documentation to WHO for emergency use consideration.

In the early stages of the study, only diagnostic kits were accessible for COVID-19 in Iran, and research authorised serologic test kits were not commercially available. Thus, based on the current kits in the recruiting phase of the study, all eligible participants needed to be negative for COVID-19 RT-PCR as well as anti-nucleocapsid IgM and IgG. After proper COVID-19 neutralising antibody detection kits were available, all the collected samples at the baseline were checked, and some samples became positive. Subsequently, a sensitivity analysis was conducted, and all participants with positive samples for neutralising antibodies in the baseline were excluded. The results of the sensitivity analysis are presented in Supplementary Appendix 3, Tables S7-S9.

Reviewer
Page 17, conclusion, please rewrite the conclusion and avoid words like 'enormously enhance' 'safety and efficacy of COVID-19 hospitalisation.
Authors
The authors appreciate the comment of the reviewer. The section was revised and now reads: Administration of the two shots of 5µg dose BIV1-CovIran vaccine with a 28-day interval would enhance the immunity of all vaccine recipients against SARS-CoV-2 with no vaccine-related SAEs. These results support further evaluation of this inactivated whole virus particle vaccine in Phase III.

VERSION 2 – REVIEW

REVIEWER	Odilon Nouatin Centre de Recherches Médicales de Lambaréné
REVIEW RETURNED	24-Jan-2022

GENERAL COMMENTS	The authors have taken into account the majority of relevant comments and suggestions.

REVIEWER	Alessandro Rovetta Mensana srls, Research and Disclosure Division
REVIEW RETURNED	23-Jan-2022

GENERAL COMMENTS	Dear authors, thank you for your replies and revisions. However, there are unclear aspects that need to be further discussed. 1) Authors response: “As we did not compare the means of more than two groups in one single test, the ANOVA test was not used in the statistical analysis.” In Appendix 2 I found this sentence: “The results of total antibody titres in the experimental groups show that injection of vaccine candidate based on One Way Analysis of Variance (ANOVA) with P<0.05 induced the level of specific antibodies on day 21 and day 42 after the first injection.” Even if these are preclinical results, it would be appropriate to specify the procedural details (e.g., how the assumptions of the analysis were verified). This can be done directly in the appendix. 2) What test or procedure was used to verify the equality of variances for the two-sample t-test? 3) Some details were explained in the responses but not included in the manuscript or supplementary files. Please add the following information to either of these two files. - “D'Agostino's K-squared test to check the normality of the distribution” - “While using the two-sample t-test, if the variance of the two groups was not equal, the Welch correction (Welch's t-test) was used.” 4) Dear authors, as argued in the references provided in the previous round (in particular, Greenland et al.), I strongly advise against the dichotomous use of the significance threshold. For example, sentences like "there were no significant differences in the incidence ratio of solicited and unsolicited AEs between the intervention and placebo groups" can be highly misleading and statistically unwarranted. Indeed, even P>.05 can provide evidence against the null hypothesis since P-values should be used as graded measures against the latter. Therefore, I suggest avoiding this type of expression. A possible alternative to "non-significance" is "low significance, limited significance" or similar. These changes should be made at all points in the manuscript where the concept of "non-significant" is adopted. 5) Some of the scientific community discouraged pre-testing for using Student or Welch t-tests as it may diminish their power (https://pubmed.ncbi.nlm.nih.gov/15171807/). Indeed, the Welch t-test can also be used in the case of equal variances. If the authors decide to keep their original approach, I suggest specifying this possible limitation. 6) Statistical significance and effect size must be kept separate. Indeed, it is possible to obtain statistically very significant results with weak effect sizes. For this reason, I suggest mentioning the extent of the effect size alongside the statistical significance (e.g., "there were low significant small differences in the incidence ratio of solicited and unsolicited AEs between the intervention and placebo groups")

	7) Authors response: “The authors appreciate the reviewer's concern. It is encouraging to compare the efficacy of vaccines of different platforms in the manuscript. Nevertheless, data from phase III studies are required for such comparison, and it could not be included in the introduction storyline of a phase I/II study. The safety and immunogenicity of various vaccine platforms are discussed in the discussion section of the manuscript. Please rest assured that we will compare the efficacy of BIV1-CovIran with other vaccines in the manuscript reporting results of phase III clinical trial. Kindly note that at the time of narration of this manuscript in June- August 2021, heterologous vaccination was not widely discussed among the scientific community. Moreover, the study presents the results of Phase I and II randomised placebo-controlled clinical trials of the BIV1-CovIran vaccine to assess its safety and immunogenicity. From where we are standing, discussing the effectiveness of heterologous vaccination is beyond the objectives of this study and could be addressed in future studies.” Dear authors, I specify that I have never said to compare the efficacy of vaccines based on different technologies but only to mention that part of the scientific literature has found greater effectiveness of mRNA and heterologous vaccinations compared to adenoviral vaccines alone against COVID-19. In particular, the introduction section provides a background on inactivated vaccines (i.e., "Inactivated vaccines have been widely used for decades and have a well-established safety profile with precise evaluation and quality control methodologies [12]. These vaccines have been used for emerging respiratory diseases and hold promise for a safe, effective, and inexpensive option against SARS-CoV-2 [13]. Notably, one inactivated viral vaccine has recently received approval for emergency use from WHO [7,14]. Furthermore, in a meta-analysis of randomized controlled clinical trials, the efficacy of inactivated vaccines against SARS-CoV-2 was reported to surpass 90% [15].") Therefore, for the background to be unbiased, it is also fair to mention that there is evidence in the literature that mRNAs and heterologous vaccinations work better against VOCs (e.g., https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(22)00094-0/fulltext, for other references see the previous round comment).

VERSION 2 – AUTHOR RESPONSE

Reviewer: 4

Dr. Alessandro Rovetta, Mensana srls

Reviewer
Dear authors, thank you for your replies and revisions. However, there are unclear aspects that need to be further discussed.

Authors
We would like to express our most sincere words of appreciation for your time and efforts regarding this manuscript. The manuscript was revised according to the comments and suggestions. We feel that the changes made according to the comments have improved the quality of the manuscript, and we would be happy, if it now meets the criteria for publication in BMJ Open.

Reviewer
1) Authors response: “As we did not compare the means of more than two groups in one single test, the ANOVA test was not used in the statistical analysis.” In Appendix 2 I found this sentence: “The

results of total antibody titres in the experimental groups show that injection of vaccine candidate based on One Way Analysis of Variance (ANOVA) with P<0.05 induced the level of specific antibodies on day 21 and day 42 after the first injection." Even if these are preclinical results, it would be appropriate to specify the procedural details (e.g., how the assumptions of the analysis were verified). This can be done directly in the appendix.

Authors

The authors agree with the meticulous comment of the reviewer. The mentioned paragraph was presented as the preclinical evidence for the clinical trial based on a study from Abdoli et al. [1]. We double-checked the text as it was presented in the preclinical study, then we revised the paragraph in Appendix 2 (the study protocol) and included a citation to the original study, which now reads:
The vaccine candidate induced the production of specific antibodies in the experimental groups in the pre-clinical study, as investigated via two-way ANOVA followed by Tukey's post hoc test.

Reviewer

2) What test or procedure was used to verify the equality of variances for the two-sample t-test?

Authors

F-test of equality of variances was used to verify the equality of variances for the two-sample t-test [2]. We also included this clarification in the statistical analysis paragraph in the methods section of the manuscript.

Reviewer

3) Some details were explained in the responses but not included in the manuscript or supplementary files. Please add the following information to either of these two files.
- "D'Agostino's K-squared test to check the normality of the distribution"
- "While using the two-sample t-test, if the variance of the two groups was not equal, the Welch correction (Welch's t-test) was used."

Authors

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

Frequency, mean, and standard deviation (SD) were used to describe the data. We used the Chi-Square test and Fisher's Exact test for categorised variables. D'Agostino's K-squared test to check the normality of the distribution [3]. F-test of equality of variances was used to verify the equality of variances for the two-sample t-test [2]. If the normality assumption was not satisfied, the means were compared using the Mann-Whitney test. In cases of normal distribution, if the variances were equal, the mean titres among groups were compared with a two-sample t-test at a two-sided 5% significance level. Otherwise, the Welch correction (Welch's t-test) was used while using the two-sample t-test.

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. Data for visualisation of weekly COVID-19 new cases and mortality in Figure 1 were derived from An interactive web-based dashboard to track COVID-19 in real-time [4].

Reviewer

4) Dear authors, as argued in the references provided in the previous round (in particular, Greenland et al.), I strongly advise against the dichotomous use of the significance threshold. For example, sentences like "there were no significant differences in the incidence ratio of solicited and unsolicited AEs between the intervention and placebo groups" can be highly misleading and statistically unwarranted. Indeed, even P>.05 can provide evidence against the null hypothesis since P-values should be used as graded measures against the latter. Therefore, I suggest avoiding this type of expression. A possible alternative to "non-significance" is "low significance, limited significance" or similar. These changes should be made at all points in the manuscript where the concept of "non-significant" is adopted.

Authors

Thank you for your comment. Amended. The corresponding sentences in the results section now read:

In Stage I, there were low significant differences in the incidence ratio of solicited and unsolicited AEs between the intervention and placebo groups.

Similar to Stage I, there were low significant differences in the incidence ratio of solicited and unsolicited AEs between intervention and placebo groups.

All AEs among the vaccinated participants in Phase I were mild or moderate, and no AESI was witnessed. There were low significant abnormalities in the laboratory assessment of participants during Phase I (Supplementary Appendix 3, Table S3 and S4).

Reviewer

5) Some of the scientific community discouraged pre-testing for using Student or Welch t-tests as it may diminish their power (https://pubmed.ncbi.nlm.nih.gov/15171807/). Indeed, the Welch t-test can also be used in the case of equal variances. If the authors decide to keep their original approach, I suggest specifying this possible limitation.

Authors

The authors agree with the meticulous comment of the reviewer. We went through the manuscript by Zimmerman. The author argued “when sample sizes are unequal, it appears that the most efficient strategy is to perform the Welch t test or a related separate-variances test unconditionally, without regard to the variability of sample values”. Kindly note that in all the two-sample t-tests performed in our study, the variances were unequal and the Welch correction (Welch's t-test) was used, except for one. While comparing the neutralising antibody level among groups receiving 3µg and 5µg of vaccine in Stage I of Phase I, the variances were equal. Thus, we performed both Welch's t-test and two-sample t-tests, the p-values of which were equal by two decimals. We hope that this has clarified the issue raised by the reviewer.

Reviewer

6) Statistical significance and effect size must be kept separate. Indeed, it is possible to obtain statistically very significant results with weak effect sizes. For this reason, I suggest mentioning the extent of the effect size alongside the statistical significance (e.g., "there were low significant small differences in the incidence ratio of solicited and unsolicited AEs between the intervention and placebo groups")

Authors

Thank you for your comment. Cramér’s V was used to investigate the effect size for the safety analysis. The corresponding measures were presented in the results section and the description was also included in the methods section.

The phrase regarding the methods section reads:

Cramér’s V was used to investigate the effect size for the safety analysis [44].

The corresponding sentences in the results section read:

In Stage I, there were low significant differences in the incidence ratio of solicited (Cramér’s V=0.46) and unsolicited (Cramér’s V=0.36) AEs between the intervention and placebo groups.

Similar to Stage I, there were low significant differences in the incidence ratio of solicited (Cramér's V=0.04) and unsolicited (Cramér's V=0.18) AEs between intervention and placebo groups.
A total number of 317 AEs occurred in 152/280 (54.0%) participants during Phase II: 125/224 (56.3%) among the 5µg group compared to 27/56 (46.4%) among the placebo group (p-value=0.23, Cramér's V=0.07). There was no difference between the incidence rates of AEs among the intervention and the placebo groups for solicited (p-value= 0.23, Cramér's V=0.07) and unsolicited (p-value=0.70, Cramér's V=0.03) AEs.

Reviewer

7) Authors response: "The authors appreciate the reviewer's concern. It is encouraging to compare the efficacy of vaccines of different platforms in the manuscript. Nevertheless, data from phase III studies are required for such comparison, and it could not be included in the introduction storyline of a phase I/II study. The safety and immunogenicity of various vaccine platforms are discussed in the discussion section of the manuscript. Please rest assured that we will compare the efficacy of BIV1-CovIran with other vaccines in the manuscript reporting results of phase III clinical trial. Kindly note that at the time of narration of this manuscript in June- August 2021, heterologous vaccination was not widely discussed among the scientific community. Moreover, the study presents the results of Phase I and II randomised placebo-controlled clinical trials of the BIV1-CovIran vaccine to assess its safety and immunogenicity. From where we are standing, discussing the effectiveness of heterologous vaccination is beyond the objectives of this study and could be addressed in future studies." Dear authors, I specify that I have never said to compare the efficacy of vaccines based on different technologies but only to mention that part of the scientific literature has found greater effectiveness of mRNA and heterologous vaccinations compared to adenoviral vaccines alone against COVID-19. In particular, the introduction section provides a background on inactivated vaccines (i.e., "Inactivated vaccines have been widely used for decades and have a well-established safety profile with precise evaluation and quality control methodologies [12]. These vaccines have been used for emerging respiratory diseases and hold promise for a safe, effective, and inexpensive option against SARS-CoV-2 [13]. Notably, one inactivated viral vaccine has recently received approval for emergency use from WHO [7,14]. Furthermore, in a meta-analysis of randomized controlled clinical trials, the efficacy of inactivated vaccines against SARS-CoV-2 was reported to surpass 90% [15].") Therefore, for the background to be unbiased, it is also fair to mention that there is evidence in the literature that mRNAs and heterologous vaccinations work better against VOCs (e.g., https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(22)00094-0/fulltext, for other references see the previous round comment).

Authors

The introduction story line was reviewed for any potential bias against various vaccine platforms. The revised introduction now reads:

A tremendous global effort has been made to rapidly produce vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as a strategy to control the coronavirus disease-2019 (COVID-19) pandemic. Experts believe that safe and effective vaccines may be a potential pathway for controlling this ongoing crisis [5,6]. Remarkably, the time between identifying SARS-CoV-2 as an emerging pathogen and completing the first clinical trial for a vaccine was less than nine months [6,7].

As of 3 August 2021, 294 vaccines were being studied, among which 110 vaccines have been tested on humans in clinical trials [8]. Fortunately, several COVID-19 vaccines showed promising results in phase 3 clinical trials, and vaccinations began in early 2021 [9,10]. World Health Organisation (WHO) has authorised emergency use for six vaccines and continues to evaluate additional proposals [11]. Nevertheless, since the introduction of vaccines against SARS-CoV-2 of various platforms worldwide, a growing body of literature has been focusing on vaccine safety [12], efficacy [13] and their estimated effectiveness [14] against infection, symptomatic and severe disease caused by SARS-CoV-2 variants, and how the effectiveness wanes over time [15].

Notwithstanding such impressive achievements, the production and distribution of billions of vaccine doses around the globe remain challenging. There are concerning inequities regarding timely access to safe COVID-19 vaccine, as only 1% of available vaccine doses worldwide have been administered in Africa. The COVID-19 Vaccines Global Access (COVAX) scheme has endeavoured to ensure fair access to vaccines, as no one is safe until everyone is safe. Nevertheless, COVAX has not progressed as expected due to the lack of support from wealthy nations and significant vaccine production challenges [16].

COVID-19 has resulted in more than 4 million reported cases and 93 thousand confirmed deaths in Iran on 6 August 2021 [17]. Since the beginning of the crisis, the Iranian healthcare system has faced limited access to life-saving medicines and equipment [18]. As of 6 August 2021, less than 3.5% of the Iranian population have been fully vaccinated for COVID-19 [17]. Considering that some 60 million adults in Iran need vaccination [19], the prompt administration of a safe domestic COVID-19 vaccine could be valuable in controlling the crisis and preventing the spread of new mutations of SARS-CoV-2.

Considering Iran's successful experiences in the mass-production of inactivated vaccines [20], efforts to make domestic vaccines of this platform against SARS-CoV-2 seemed feasible. BIV1-CovIran is an inactivated whole virus particle vaccine that has demonstrated safety and immunogenicity in preclinical studies in mice, rabbits, and non-human primates [21]; therefore, it was approved for progression to human studies. This study presents the results of Phase I and II randomised placebo-controlled clinical trials of the BIV1-CovIran vaccine to assess its safety and immunogenicity.

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Reviewer: 3

Dr. Odilon Nouatin, Centre de Recherches Médicales de Lambaréné
Comments to the Author:
The authors have taken into account the majority of relevant comments and suggestions.
Authors
We would like to express our most sincere words of appreciation for your time and efforts regarding this manuscript.

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

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

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

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

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

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

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

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

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

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