A phase 3, randomised, double-blind, placebo-controlled clinical trial evaluation of the efficacy and safety of a SARS-CoV-2 recombinant spike RBD protein vaccine in adults (ABDALA-3 study)

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Summary

Background The pandemic of COVID-19 raised the urgent need for safe and efficacious vaccines against SARS-CoV-2. We evaluated the efficacy and safety of a new SARS-CoV-2 virus receptor-binding domain (RBD) vaccine.

Methods A phase 3, multicentre, randomised, double-blind, placebo-controlled trial was carried out at 18 clinical sites in three provinces of the south-eastern region of Cuba. Subjects (healthy or those with controlled chronic diseases) aged between 19 and 80 years, who gave written informed consent were eligible. Subjects were randomly assigned (1:1, in blocks) to two groups: placebo, and 50 μg RBD vaccine (Abdala). The product was administered intramuscularly, 0.5 mL in the deltoid region, in a three-dose immunization schedule at 0-14-28 days. The organoleptic characteristics and presentations of the vaccine and placebo were identical. All participants (subjects, clinical researchers, statisticians, laboratory technicians, and monitors) remained blinded during the study period. The main endpoint was to evaluate the efficacy of the Abdala vaccine in the prevention of symptomatic COVID-19. The trial is registered with the Cuban Public Registry of Clinical Trials, RPCEC00000359.

Findings Between March 22 to April 03, 2021, 48,290 subjects were included (24,144 and 24,146 in the placebo and Abdala groups, respectively) in the context of predominant D614G variant circulation. The evaluation of the main efficacy outcomes occurred during May–June 2021, starting at May 3rd, in the context of high circulation of mutant viruses, predominantly VOC Beta. The incidence of adverse reactions for individuals in the placebo and Abdala vaccine groups were 1227/24,144 (5.1%) and 1621/24,146 (6.7%), respectively. Adverse reactions were mostly mild, and from the injection site, which resolved in the first 24–48 h. No severe adverse events with demonstrated cause–effect relationship attributable to the vaccine were reported. Symptomatic COVID-19 disease was confirmed in 142 participants in the placebo group (78.44 incidence per 1000 person-years, 95% confidence interval [CI], 66.07–92.46) and in 11 participants in Abdala vaccine group (6.05 incidence per 1000 person years; 95% CI 3.02–10.82). The Abdala vaccine efficacy against symptomatic COVID-19 was 92.28% (95% CI 85.74–95.82).

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Moderate/serious forms of COVID-19 occurred in 30 participants (28 in the placebo group and only 2 in the Abdala vaccine group) for a vaccine efficacy of 92.88% (95% CI 70.12–98.31). There were five critical patients (of which four died), all in the placebo group.

Interpretation The Abdala vaccine was safe, well tolerated, and highly effective, fulfilling the WHO target product profile for COVID-19 vaccines. Those results, along with its immunization schedule and the advantage of easy storage and handling conditions at 2–8 °C, make this vaccine an option for the use in immunization strategies as a key tool for the control of the pandemic.

Research in context

Evidence before this study
We searched PubMed website on February 27, 2023, for published research articles, with no language restrictions, using the search terms "COVID-19", "SARS-CoV-2", "vaccine" and "efficacy". We found 3490 results that were restricted to 69 by the addition of "phase 3 clinical trial". In general, the articles included results of different efficacy trials of vaccines already authorized and widely used, and different technologies mostly mRNA, inactivated vaccines and non-replicating viral vectors, among others. Although several subunit vaccines based on RBD protein are currently in advanced phases of clinical development, we found few phase 3 publications regarding vaccine efficacy: Novavax/NVXCoV2373 (full length recombinant S protein-micelle nanoparticle/matrix M adjuvant) 89.7% (95% CI 80.2–94.6), Soberana-02/Soberana-Plus (heterologous three doses combination of recombinant RBD conjugated to tetanus toxoid/RBD-dimer in Alum) 92.0% (95% CI 80.4–96.7), and ZF2001 vaccine, based in a tandem-repeat dimeric RBD of the SARS-CoV-2 spike protein that is produced in Chinese hamster ovary cells and then adjuvanted with aluminium hydroxide, 81.4% (95% CI 73.3–87.3).

Even after the distribution of millions of doses of different approved COVID-19 vaccines all over the world, there is still a global need for new vaccines to control the pandemic, by increasing availability as well as better storage and distribution conditions.

Added value of this study
We previously reported the Phase 1–2 clinical trial of Abdala vaccine in adults from 19 to 80 years of age, using a three-dose immunization schedule at 0-14-28 days. The vaccine was well tolerated and no safety concerns were raised. High anti-RBD IgG immune responses were elicited as well as neutralizing antibodies against SARS-CoV-2. In this phase 3, randomised, double-blind, placebo-controlled clinical trial in 48,290 participants using the same immunization schedule, we confirmed the satisfactory safety profile previously found, but now in a larger population of individuals where around 74% had some previous coexisting illness conditions. Abdala vaccine efficacy against symptomatic COVID-19 was 92.28% (95% CI 85.74–95.82) and 92.88% (95% CI 70.12–98.31) against moderate/serious forms of the disease. There were five critical patients (of which four died), all in the placebo group. The efficacy was evaluated in the context of high circulation of mutant viruses, predominantly VOC Beta.

In this phase 3 trial, we demonstrated for the first time the safety and efficacy of a COVID-19 vaccine based on a SARS-CoV-2 recombinant spike RBD protein produced in the yeast *Pichia pastoris*, with a very well-known technology platform that we have been used for the large-scale production of hepatitis B vaccine in the last 30 years.

Implications of all the available evidence
Abdala vaccine is safe and highly protective against symptomatic COVID-19, including the most severe forms of the disease and death, fulfilling the WHO target product profile for COVID-19 vaccines. Those results, along with its compact three doses immunisation schedule, the advantages that offer the use of a very well-established technology platform, the demonstrated vaccine thermo stability and the easy storage and handling conditions at 2–8 °C, make this affordable vaccine an option for the use in massive immunisation strategies, that can contribute to worldwide efforts in the control of the pandemic.

Introduction
The causal agent of the COVID-19 pandemic is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 21 August 2022, 593 million confirmed cases and 6.4 million deaths had been reported globally, according to data recorded by the World Health Organization (WHO).[1]

There is consensus that, as long as no safe and effective preventive vaccines are available for SARS-

CoV-2, in quantities sufficient to implement comprehensive immunization programmes, the world will not return to normality. Vaccines are urgently needed to mitigate the consequences of this pandemic and protect humanity from future epidemics caused by this virus.[2]

The Cuban COVID-19 Abdala vaccine (from now onwards Abdala) was designed at the Genetic Engineering and Biotechnology Centre (CIGB) in Havana, Cuba, considering the state-of-the-art research on COVID-19 vaccine, especially the immunological aspects necessary for the development of vaccines against this disease. The vaccine antigen is based on the recombinant receptor-binding domain (RBD) subunit of the SARS-CoV-2 spike protein, produced in *P. pastoris* yeast, adjuvanted to alumina.[3,4] This is a very well-known technology platform previously used by this institution.

An exploratory phase 1-2 study, carried out in adults 19–80 years of age, evaluated Abdala's safety and immunogenicity. It was safe, well-tolerated and induced strong humoral immune responses against SARS-CoV-2.[5]

The aim of this study was to evaluate Abdala's efficacy and safety in preventing symptomatic disease due to SARS-CoV-2 infection in adults, 19–80 years of age.

Methods
Study design
A phase 3, multicentre, randomised, double-blind, placebo-controlled clinical trial was carried out at 18 clinical sites in three provinces of the south-eastern region of Cuba.

The trial was conducted in medical wards and certified areas for the vaccination process. The clinical researchers selected the participants in the trial. They were specialists in family medicine, internal medicine and intensive care, and were also in charge of clinical and safety evaluations, as well as the proper collection of the primary information generated in the study. Trial on-site monitoring (by the promoter centre monitors) verified this process as well as the accuracy of all case report forms, and all Good Clinical Practices procedures. Data management was carried out by direct supervision of specialized and independent engineers. All statistical analyses were performed by independent statisticians at the Cybernetics, Mathematics and Physics Institute, in Havana. An independent data monitoring committee (constituted with one of the following independent specialists: statistician, pathologist, clinician, epidemiologist and pharmacist) was established before starting the study to assesses safety and primary trial data, to make timely recommendations to the sponsor. The protocol followed the Helsinki Declaration Guidelines and was evaluated by an *ad hoc* Centralized Ethics Committee, integrated by members of the Ethics and Review Committee of the "Saturnino Lora" Provincial Hospital in Santiago de Cuba (main clinical site),

extended with Research Ethics Committees members from the Universities of Medical Sciences of Santiago de Cuba, Guantánamo and Granma provinces, who granted ethical approval of the study (approval No 1/2021). This Review Board was constituted with highly qualified medical specialists not linked to the study, as well as a member of the community. This committee made the research following-up, ensuring the protection of the rights, safety and well-being of the participants in the study. Additionally, the Cuban Centre for State Control of Medicines, Equipment and Medical Devices (CECMED) approved the beginning of the clinical trial after considering the scientific, methodological and ethical aspects. The manuscript adheres to CONSORT reporting guidelines.

Participants
The study universe included adults of any sex, with permanent residence in the capital cities of Santiago de Cuba, Guantánamo and Granma provinces, who responded to the call for the study. Once the selection criteria were verified, the subjects were included in the trial and identified with a code including a consecutive number. Subjects aged from 19 to 80 years (healthy adults or with controlled comorbidities), who signed their written informed consent to participate, were enrolled. Exclusion criteria were: subjects with previous SARS-CoV-2 infection confirmed by reverse-transcriptase polymerase chain reaction (RT-PCR) or at the moment of inclusion; individuals suspected to have the infection or contact with a COVID-19 case, or previous acute infections in the last 15 days; those with uncontrolled chronic diseases at the time of inclusion; subjects who had received a vaccine candidate against COVID-19 or with any medical condition that required an immunomodulator, systemic steroid or cytostatic during the study; Body Mass Index ≤ 18 or ≥ 35 kg/m^2; subjects with tattoos on both deltoid regions that interfered with the local safety assessment; those who received blood, blood products, or any investigational product in the last three months; subjects with known hypersensitivity to thiomersal and to any component of the formulation under study; history or suspicion of alcoholism or drug dependence; pregnancy or lactation; mental disability to issue consent.

Randomisation and masking
The study subjects were randomly distributed (1:1) into 2 groups: I) placebo and II) 50 µg RBD (Abdala vaccine). Randomisation was carried out at the CIGB's Clinical Research Direction, in blocks of 4 individuals, for each clinical site, by means of a computerised random number generator. The clinical sites received the product (Abdala and placebo) in such blocks, in masked vials, to avoid their identification. Their organoleptic characteristics and presentations were identical. As a result, the decision to accept or reject a participant was

made without knowing the assignment in the sequence. The trial participants, researchers, and monitors, were unaware of the trial-group assignments during the whole trial. Statistical analyses were done without the knowledge of the group's identity. This was known after the analyses were concluded.

Procedures

Abdala is a slightly opaque greyish-white suspension. After a settling time it separates into two phases: the upper one is a transparent liquid and the lower is a precipitated gel, easily resuspended after shaking and is easily resuspended and is essentially free of foreign particles. Each mL contains 100 µg RBD (active ingredient), 0.60 mg aluminium hydroxide gel, 0.56 mg disodium hydrogen phosphate, 0.62 mg sodium dihydrogen phosphate dihydrate, 8.5 mg sodium chloride and 0.05 mg thiomersal (antimicrobial). Placebo vial had the same excipients, except the RBD. Their organoleptic characteristics and presentations were identical.

Preparation and administration of both formulations was done by personnel trained for these purposes, under the researcher indication and supervision. Each clinical site had a stock with the masked products (Abdala and placebo), stored at 2–8 °C, in quantity sufficient to ensure the progress of the study protocol without interruptions due to this fact. The sponsor was responsible for the continuous supply of the products.

The products (Abdala or placebo) were administered intramuscularly, 0.5 mL in the deltoid region, in a three-dose immunization schedule at 0-14-28 days.

Symptomatic COVID-19 cases were defined as participants with a nasopharyngeal swab positive for SARS-CoV-2 by RT-PCR, who presented at least one major symptom or sign (new onset dyspnoea or worsening, oxygen saturation [SpO_2] ≤92% by pulse oximetry without oxygen supplement, persistence of chest pain, change of behaviour or alteration in the state of consciousness, local or generalized cyanosis or pneumonia by clinical or imaging diagnosis) or at least two minor symptoms or signs (fever ≥38 °C, headache, chills, odynophagia, myalgia, fatigue that interferes with daily activity, vomiting and/or diarrhoea, anosmia and/or ageusia).

RT-PCR SARS-CoV-2 positive cases, detected in nasopharyngeal swabs at the Hygiene, Epidemiology and Microbiology Provincial Centres, were notified to the research team at clinical sites participating in the trial. SARS-CoV-2 infection was diagnosed using RIDA®GENE SARS-CoV-2 (R-Biopharm AG, Germany). Cases were confirmed using the LightMix Modular SARS-CoV-2 (COVID19) RdRP-gene (TIB Molbiol/Roche Diagnostics, Germany).

Cases were also captured by the spontaneous notification of the volunteers through an emergency 24-h telephone number to contact the main researcher. Additionally, as part of the epidemiological strategies of

the Cuba's Ministry of Public Health for the identification of new COVID-19 cases and their contacts, an active daily search by family doctors was also in place that allowed the identification of new cases among the volunteers through their identity card as participants in the trial. All positive cases were immediately sent to the "Joaquín Castillo Duany" Hospital in Santiago de Cuba, as the centralized care unit for all the COVID-19 patients in the trial, who remained hospitalized for a better follow-up.

Clinical data were judged by an independent adjudication committee of medical doctors at the hospital, which was unaware to which study group the patient was assigned. Clinical forms of symptomatic COVID-19 disease were classified into four categories: mild, moderate, serious and critical disease, following the same international criteria used when addressing the clinical spectrum of SARS-CoV-2 infection.[6]

Efficacy analyses were carried out in individuals without evidence of SARS-CoV-2 exposure before the first dose of the immunization schedule ("modified intention to treat" [mITT] and "per protocol" [PP] populations). For that purpose, serum samples (taken at basal time from all the participants in the trial) were tested using the validated UMELISA ANTI SARS-CoV-2, a qualitative assay for the detection of total antibodies against SARS-CoV-2 in human serum or plasma (manufactured by Immunoassay Centre, Havana. Registration number D2010-42, CECMED).

Adverse events (AE) were carefully registered according to the type, duration, severity, outcome and causality relationship. AE's severity was classified into five levels: (1) mild, if no therapy was necessary; (2) moderate, if a specific treatment was needed; (3) severe or medically significant but not immediately life-threatening; cause hospitalization or prolongation of hospitalization; disabling; (4) life-threatening, requires urgent intervention and (5) AE-related death.[7] A qualitative assessment was used to classify the causal relationship following WHO recommendation.[8] Adverse reactions (AR) expected with vaccination were especially sought (pain at the injection site, erythema, induration, headache, fever, among others).

All participants were evaluated in the first hour after the administration of each dose by anamnesis, vital signs (temperature, blood pressure, respiratory and cardiac frequencies), an inspection of the injection site (to detection local symptoms) as well as general physical examination. Subsequently, an active/passive surveillance of AE was in place for the 14 days-period between each dose, combining home visits of family physicians at the community level (within 72 h after each dose) with the self-reporting of any AE that may occur throughout the trial.

Outcomes

The primary endpoint of the trial was to evaluate Abdala's efficacy in preventing the first occurrence of

symptomatic COVID-19, in individuals without evidence of prior exposure to SARS-CoV-2 infection with onset 14 days after the third dose of the immunization schedule (PP population). The consistency of vaccine efficacy (VE) was evaluated in various subgroups, including sex, age groups, ethnicity and comorbidities. Secondary endpoints were the prevention of mild and moderate/serious forms of COVID-19 (in PP population) as well as the prevention of symptomatic COVID-19 cases that occurred in the subset of individuals analysed by "intention-to-treat" (ITT) without evidence of prior exposure to SARS-CoV-2 infection at the time of inclusion in the study, before the first dose of the investigational product (mITT population). Safety outcome included the collection of AE in all enrolled volunteers that received at least one dose of the vaccine or placebo (ITT population).

Statistical analysis

The sample size was calculated considering the total number of cases needed to demonstrate Abdala's VE to prevent symptomatic COVID-19. Assuming proportional risks over time with 1:1 randomization of vaccine and placebo groups, a total of 151 cases of COVID-19 would be needed to detect a 60% reduction in the hazard rate (i.e., 60% VE) with 30% as the lower limit of 95% confidence interval (95% CI) to reject the null hypothesis H_0: VE $\leq$ 30% with a statistical power of 0.90. Based on the estimated incidence rate of 0.286% in two months for the placebo group in the study sites, the enrolment of 48,000 participants was required, anticipating a 2% dropout rate. Details of the sample size calculation are shown in the study protocol (Appendix).

Two interim analyses were planned. Supplemental Material (S3, Table S3).

Participants' data were collected at the time of symptom onset and censored at the end of their follow-up. In each analysis, non-parametric estimates of the survival functions of the control and experimental groups were calculated using the Kaplan–Meier method for censored data. Graphs of the cumulative hazard functions were obtained. Log-rank hypothesis tests were performed to check for differences between the survival curves of the study groups. VE, defined as the percentage reduction in the hazard ratio, was calculated by the expression VE = 1-HR, where HR represents the ratio between the hazard rate functions of the experimental and control groups. This ratio was estimated by Cox regression models for proportional hazards. For the primary endpoint, the Cox regression was stratified by age groups, from 19 to 50 years and from 51 to 80, as planned in the protocol. To model mild and moderate/serious cases, multi-state Cox regression was used. The validation of the Cox models was done by analysing their respective residual graphs and testing their Schoenfeld residuals.

To assess safety, AEs were tabulated and plotted by study group. AEs' incidence rates in each group were described in the ITT population, defined as the total cohort of participants who received at least one injection, and considered in the group where they were randomized.

The mITT population was defined as the subset of individuals in the ITT definition that showed no previous immunologic or virological evidence of COVID-19 infection at the time of study inclusion, prior to the first dose of the investigational product. This population was used for the secondary outcome of VE.

The primary efficacy end point in the interim and primary analyses was assessed in the PP population, defined as the subset of volunteers included, who met all the eligibility criteria, with no previous immunologic or virological evidence of COVID-19 infection at the time of study inclusion, prior to the first dose of the investigational product, who received the complete three-dose immunization schedule, in whom the primary endpoint assessment was available and who did not have any major protocol deviations. Participants were considered in the group where they were randomized. Subgroup analyses were performed by baseline characteristics, including age, sex, ethnicity and presence or absence of coexisting conditions, also carried out in the PP population. Subgroups analyses were based on any symptomatic infection. Incidence rates per 1000 person-years were also calculated.

Statistical analyses were conducted using R version 3.6.2. The input of the primary information in databases built with the OpenClinica software (www.openclinica.com), was performed remotely (from the clinical site) and in duplicate (independently by two operators) for subsequent automatic comparison and correction of the databases, necessary for statistical analysis with accurate trial information. Access to these databases at the CIGB (with a user name and password) was made through the national health system network (INFOMED). For debugging errors, data not matching the one recorded in the original forms were corroborated, to avoid confusion.

This study was registered in the Cuban Public Clinical Trial Registry, RPCEC00000359.

Role of the funding source

The funder of the study had a role in study design, data interpretation, and writing of the report, but had no role in data collection or data analysis.

Results

From 22 March to 03 April, 2021, a total of 48,290 subjects were included out of 49,656 that were screened in the context of predominantly SARS-CoV-2's D614G variant circulation. Their disposition is shown in Fig. 1. Subjects were randomly distributed into two study

Not included: **1366**
Uncontrolled chronic disease: 593
Body mass index ≤18 or ≥35 kg/m^2: 392
Immunomodulatory or steroidal
treatment in the last 3 months: 112
Acute infection in the last 15 days: 60
Pregnancy or lactation: 44
Thiomersal allergy: 30
Subject refused: 28
Physical examination with
significant alterations: 17
Subject with Age < 19 or >80 years: 15
SARS-CoV-2 positive RT-PCR: 7
Other: 68

49656 subjects screened

48290 randomised

Placebo (24144 subject)

Abdala vaccine (24146 subject)

1st dose: 24144 received

1st dose: 24146 received

Withdrawal: **318**
Voluntary dropout: 197
SARS-CoV-2 infection: 39
Non-adherence to the protocol: 38
Uncontrolled chronic disease: 23
Pregnancy: 11
Death (stomach adenocarcinoma): 1
Other (less frequently): 9

Withdrawal: **352**
Voluntary dropout: 229
Non-adherence to the protocol: 47
SARS-CoV-2 infection: 41
Uncontrolled chronic disease: 19
Pregnancy: 11
Death (cerebral hemorrhage;
suicide by hanging; COVID-19): 3
Other (less frequently): 2

2nd dose: 23826 received

2nd dose: 23794 received

Withdrawal: **224**
Voluntary dropout: 113
SARS-CoV-2 infection: 55
Non-adherence to the protocol: 25
Pregnancy: 12
Uncontrolled chronic disease: 9
Death (severe cardiac arrhythmia;
generalized sepsis; acute myocardial
infarction; severe ventricular arrhythmia): 4
Other (less frequently): 6

Withdrawal: **237**
Voluntary dropout: 156
SARS-CoV-2 infection: 36
Non-adherence to the protocol: 31
Uncontrolled chronic disease: 7
Pregnancy: 3
Death (hypostatic pneumonia;
bilateral massive bronchopneumonia): 2
Other (less frequently): 2

3rd dose: 23602 received

3rd dose: 23557 received

- 24144 included in safety analysis (intention to treat).
- 20157 included in modified intention-to-treat efficacy analysis*.
- 19674 included in "per protocol" analysis of efficacy.

Causes of exclusion of PP analysis**:
- Voluntary dropout: 310
- SARS-CoV-2 infection: 116
- Excluded from the study due to non-adherence to the protocol: 63
- Uncontrolled chronic disease: 32
- Pregnancy: 23
- Death of subject: 5
- Other (less frequently): 15

- 24146 included in safety analysis (intention to treat).
- 20195 included in modified intention-to-treat efficacy analysis.
- 19689 included in "per protocol" analysis of efficacy.

Causes of exclusion of PP analysis**:
- Voluntary dropout: 385
- SARS-CoV-2 infection: 83
- Excluded from the study due to non-adherence to the protocol: 78
- Uncontrolled chronic disease: 26
- Pregnancy: 14
- Death of subject: 5
- Other (less frequently): 4

* one individual was excluded of the efficacy analysis in mITT population, considering that it was not possible to specify precisely the date of onset of the symptoms after the second dose of the immunization schedule.

** There are 169 subjects (80 and 89 in the Placebo and Abdala groups, respectively) that discontinued the trial for various reasons (counting in the causes of exclusion from the PP analysis), but also had total antibodies against SARS-CoV-2 at baseline (consequently discounted from the mITT population). In each efficacy analysis the participant counted only once.

Fig. 1: **Study flow chart**.

groups of the vaccination schedule (0-14-28 days): placebo and Abdala (RBD 50 μg). All volunteers completed the vaccination schedule (three doses), except for 1131 subjects that discontinued vaccination (542 in the placebo group, and 589 in Abdala group). Fig. 1 also summarizes individuals, by cause, who discontinued the 2nd and 3rd administration of the investigational product. The main cause of withdrawal was voluntary dropout which occurred in 61.5% (695/1131) of the participants. Other causes were: SARS-CoV-2 infection, non-adherence to the protocol by subjects (optional decision provided in the research protocol) and uncontrolled chronic diseases, among others. Considering all subjects included, high compliance with the planned vaccination was observed (97.7%).

Table 1 shows the demographic and baseline characteristics of participants. Most of them were women (25,286, 52.4%); mean (±SD) age was 48.9 ± 16.2 years.

Overall, 52.2% (25,186) were mestizo, 26.1% (12,605) white and 21.7% (10,499) black, coinciding with the ethnic distribution of the Cuban population in the south-eastern region of the country where the study was conducted. No relevant imbalances between the study groups were observed. Supplemental Material (S1, Table S1) shows the demographic characterization of these individuals by age ranges.

Seventy-four per cent of participants had some co-morbidity or risk factor, with a similar distribution in both study groups. The most frequent were overweight/type I obesity, hypertension, smoking, diabetes mellitus and bronchial asthma, being of interest due to their clinical relevance in relation to COVID-19 (Table 1). The population involved in this Abdala's efficacy clinical trial was very close to real-world conditions.[9]

Only 19% of subjects reported some AE; 15,937 AEs were reported of 150 different types. AEs' frequency by

Variable	Placebo	Abdala vaccine	Total
N (%)	24,144 (50.0)	24,146 (50.0)	48,290 (100)
Sex – no. (%)			
Female	12,689 (52.6)	12,597 (52.2)	25,286 (52.4)
Male	11,455 (47.4)	11,549 (47.8)	23,004 (47.6)
Age (years)			
Means ± SD	48.9 ± 16.2	48.9 ± 16.1	48.9 ± 16.2
Median (IQR)	50 (15)	50 (15)	50 (15)
Ethnicity– no. (%)			
White	6248 (25.9)	6357 (26.3)	12,605 (26.1)
Black	5233 (21.7)	5266 (21.8)	10,499 (21.7)
Mestizo	12,633 (52.4)	12,523 (51.9)	25,186 (52.2)
BMI (kg/m^2)			
Means ± SD	26.5 ± 4.4	26.5 ± 4.3	26.5 ± 4.3
Median (IQR)	26.4 (6.2)	26.4 (6.2)	26.4 (6.2)
Subjects with some comorbidity – no. (%)	17,928 (74.3)	17,931 (74.3)	35,859 (74.3)
Main comorbidities/risk factors – no. (%)			
Overweight/type I obesity	11,806 (48.9)	11,756 (48.7)	23,562 (48.8)
High blood pressure	8223 (34.1)	8261 (34.2)	16,484 (34.1)
Smoking	6339 (26.3)	6197 (25.7)	12,536 (26.0)
Diabetes mellitus	1959 (8.1)	1945 (8.1)	3904 (8.1)
Bronchial asthma	1881 (7.8)	1928 (8.0)	3809 (7.9)
Heart disease	897 (3.7)	897 (3.7)	1794 (3.7)
Other CNS and peripheral disorders	878 (3.6)	899 (3.7)	1777 (3.7)
Allergy	852 (3.5)	853 (3.5)	1705 (3.5)
Gastrointestinal disorders	789 (3.3)	799 (3.3)	1588 (3.3)
COPD	259 (1.1)	243 (1.0)	502 (1.0)
Cancer	250 (1.0)	227 (0.9)	477 (1.0)
Ischemic/hemorrhagic cerebral infarction	151 (0.6)	163 (0.7)	314 (0.7)
Genitourinary disorders	149 (0.6)	132 (0.5)	281 (0.6)
Disorders of red blood cells and platelets	85 (0.4)	82 (0.3)	167 (0.3)
Deep and superficial vein thrombosis	28 (0.1)	36 (0.1)	64 (0.1)
Subjects with total antibodies against SARS-CoV-2 at baseline	3986 (16.5)	3951 (16.4)	7937 (16.4)

BMI: Body-mass index (the weight in kilograms divided by the square of the height in meters. The calculation was based on the weight and height measured at the time of screening); CNS: Central Nervous System; COPD: Chronic Obstructive Pulmonary Disease; IQR: Interquartile range; SD: standard deviation.

Table 1: Demographic and baseline characteristics of the participants in the ABDALA-3 study at enrolment.

affected organ systems, according to dose and study group is presented in Supplemental Material (S2, Table S2). With consistent causality to vaccination, 21.8% (1933 AEs) were reported in the 1st dose, 22.9% (1214) in the 2nd dose and 23.5% (417) in the 3rd dose. The remaining AEs were not related to the formulations under study or their cause–effect relationship was indeterminate. Table 2 characterizes the AEs at each dose, by study group.

Abdala was well tolerated. The overall incidence of AR was 1227/24,144 (5.1%) for individuals in the placebo group and 1621/24,146 (6.7%) for Abdala group. Reactogenicity was largely absent or mild in most reports, and next doses were neither withheld nor delayed for this cause (Fig. 2).

Depending on the number of doses administered, reactogenicity was very low: 1480 events in 71,572 doses administered (2.1%) in the placebo group, and 2084/71,497 (2.9%) in the Abdala group, with a huge predominance (>97%) of local AR, mainly pain at the injection site, which represented 84.3% of the total

reported. These reports were similar between study groups, slightly higher in the Abdala group. More than half of these AR were reported during the observation period corresponding to the first dose and were significantly reduced in the following doses. Most of the AR resolved spontaneously in the first 24–48 h without medication.

No severe AEs with demonstrated cause–effect relationship attributable to the investigational product were reported and there were no withdrawals for this cause. No episodes of anaphylaxis, immune-mediated medical conditions, myocarditis as well as other events of special interest relevant to COVID-19 were reported in this research.

During the vaccination period, a total of 10 serious AEs leading to death occurred in five participants in the placebo group, and five subjects in the Abdala group, described in Fig. 1. No deaths were considered by researchers to be related to the vaccine or placebo. Subsequently, during the evaluation of the VE (from 14 days after completion of the vaccination schedule), there were

Variable	DOSE 1			DOSE 2			DOSE 3		
	Placebo	Abdala	Total	Placebo	Abdala	Total	Placebo	Abdala	Total
N	24,144	24,146	48,290	23,826	23,794	47,620	23,602	23,557	47,159
Subjects with adverse events	3000 (12.4%)	3177 (13.2%)	6177 (12.8%)	1749 (7.3%)	1983 (8.3%)	3732 (7.8%)	579 (2.5%)	639 (2.7%)	1218 (2.6%)
Total of adverse events (15,937)	4318 (48.7%)	4545 (51.3%)	8863 (55.6%)	2442 (46.0%)	2861 (54.0%)	5303 (33.3%)	838 (47.3%)	933 (52.7%)	1771 (11.1%)
Intensity									
Mild	3942 (91.3%)	4166 (91.7%)	8108 (91.5%)	2208 (90.4%)	2598 (90.8%)	4806 (90.6%)	719 (85.8%)	781 (83.7%)	1500 (84.7%)
Moderate	371 (8.6%)	366 (8.1%)	737 (8.3%)	230 (9.4%)	260 (9.1%)	490 (9.2%)	114 (13.6%)	150 (16.1%)	264 (14.9%)
Severe (Grade 3)	2 (0.04%)	1 (0.02%)	3 (0.03%)	0	0	0	5 (0.6%)	2 (0.2%)	7 (0.4%)
Serious (Grade 4)	2 (0.04%)	1 (0.02%)	3 (0.03%)	0	1 (0.03%)	1 (0.01%)	0	0	0
Serious (Grade 5)	1 (0.02%)	3 (0.07%)	4 (0.05%)	4 (0.2%)	2 (0.07%)	6 (0.1%)	0	0	0
Causality (WHO)									
Inconsistent with vaccination	2871 (66.5%)	2849 (62.7%)	5720 (64.5%)	1632 (66.8%)	1746 (61.0%)	3378 (63.7%)	588 (70.2%)	561 (60.1%)	1149 (64.9%)
Indeterminate	596 (13.8%)	614 (13.5%)	1210 (13.7%)	334 (13.7%)	377 (13.2%)	711 (13.4%)	97 (11.6%)	108 (11.6%)	205 (11.6%)
Consistent with vaccination	851 (19.7%)	1082 (23.8%)	1933 (21.8%)	476 (19.5%)	738 (25.8%)	1214 (22.9%)	153 (18.3%)	264 (28.3%)	417 (23.5%)
Action taken									
None	2817 (65.2%)	3025 (66.6%)	5842 (65.9%)	1629 (66.7%)	1962 (68.6%)	3591 (67.7%)	584 (69.7%)	628 (67.3%)	1212 (68.4%)
Non-drug therapy	132 (3.1%)	133 (2.9%)	265 (3.0%)	44 (1.8%)	52 (1.8%)	96 (1.8%)	9 (1.1%)	16 (1.7%)	25 (1.4%)
Drug therapy	1372 (31.8%)	1385 (30.5%)	2757 (31.1%)	762 (31.2%)	842 (29.4%)	1604 (30.2%)	241 (28.8%)	289 (31.0%)	530 (29.9%)
Hospitalization	1 (0.02%)	0	1 (0.01%)	3 (0.1%)	0	3 (0.05%)	4 (0.5%)	0	4 (0.2%)
Withdrawal from study	6 (0.1%)	11 (0.2%)	17 (0.2%)	4 (0.2%)	5 (0.2%)	9 (0.2%)	0	0	0
Outcome									
Completely resolved	4277 (99.1%)	4499 (99.0%)	8776 (99.0%)	2421 (99.1%)	2845 (99.4%)	5266 (99.3%)	822 (98.1%)	919 (98.5%)	1741 (98.3%)
Resolved with sequelae	4 (0.09%)	4 (0.09%)	8 (0.09%)	2 (0.08%)	2 (0.07%)	4 (0.07%)	0	0	0
Getting better	28 (0.6%)	27 (0.6%)	55 (0.6%)	11 (0.5%)	9 (0.3%)	20 (0.4%)	12 (1.4%)	10 (1.1%)	22 (1.2%)
Present and unchanged	8 (0.2%)	11 (0.2%)	19 (0.3%)	4 (0.2%)	3 (0.1%)	7 (0.1%)	4 (0.5%)	4 (0.4%)	8 (0.5%)
Worsening	0	1 (0.02%)	1 (0.01%)	0	0	0	0	0	0
Death from adverse event	1 (0.02%)	3 (0.07%)	4 (0.05%)	4 (0.2%)	2 (0.07%)	6 (0.1%)	0	0	0

The variable Action taken was tabulated following a hierarchical structure, to ensure that its categories are exclusive. Levels of this variable are ordered from least to most "drastic" measures: None, Non-pharmacological therapy, Pharmacological therapy, Hospitalization and Withdrawal from the study.

Table 2: Overall distribution and characterization of adverse events, according to dose and study group (reported from the beginning of the study until 14 days after completing the vaccination schedule).
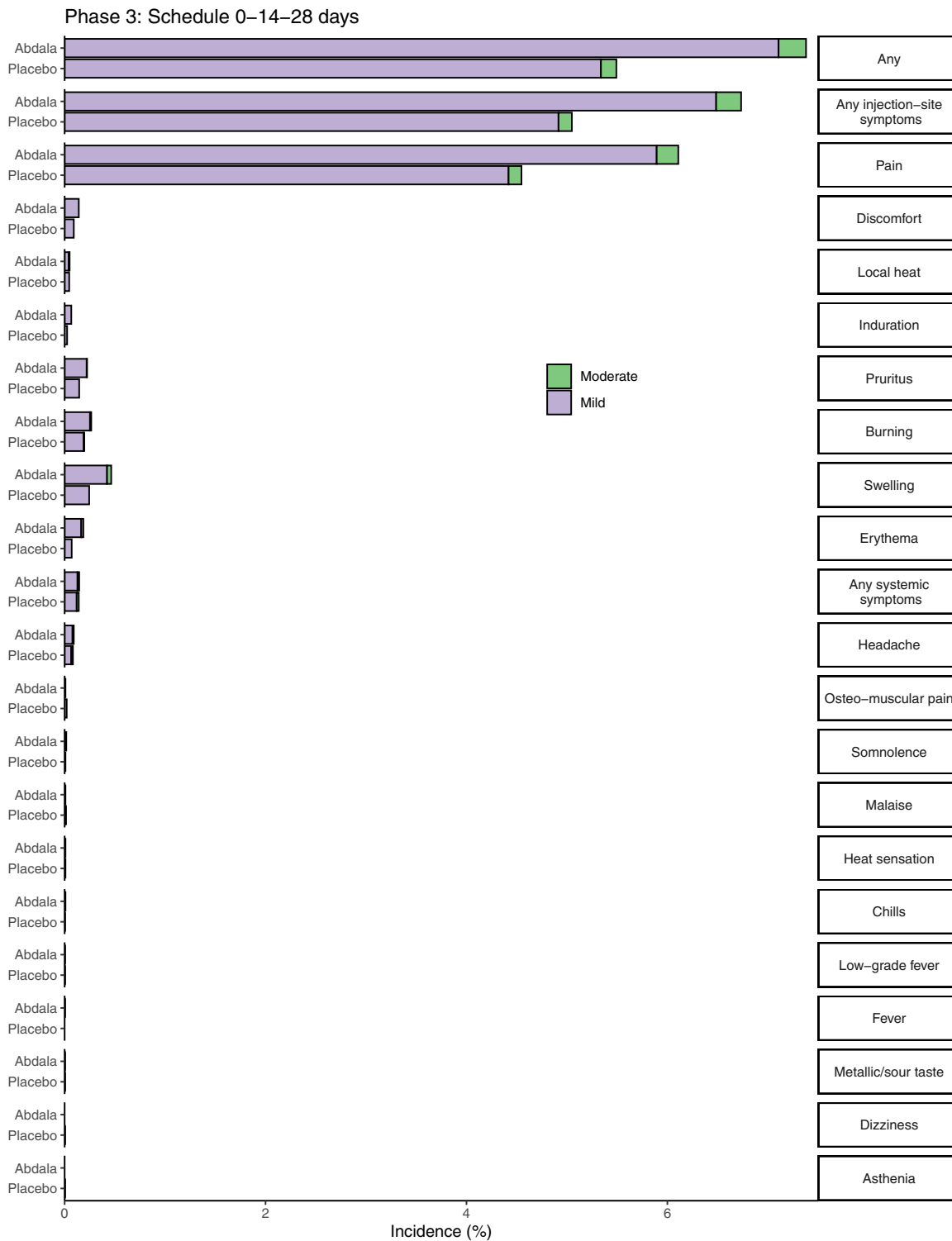
Fig. 2: Percentage of participants according to the occurrence of adverse reactions, by study group. The percentage of participants in each study group (Abdala vaccine, Placebo) with adverse reactions according to the maximum toxicity grade (mild or se) from first dose up to 14 days after the third dose is plotted by signs or symptoms. Participants who reported 0 events make up the remainder of the 100%.
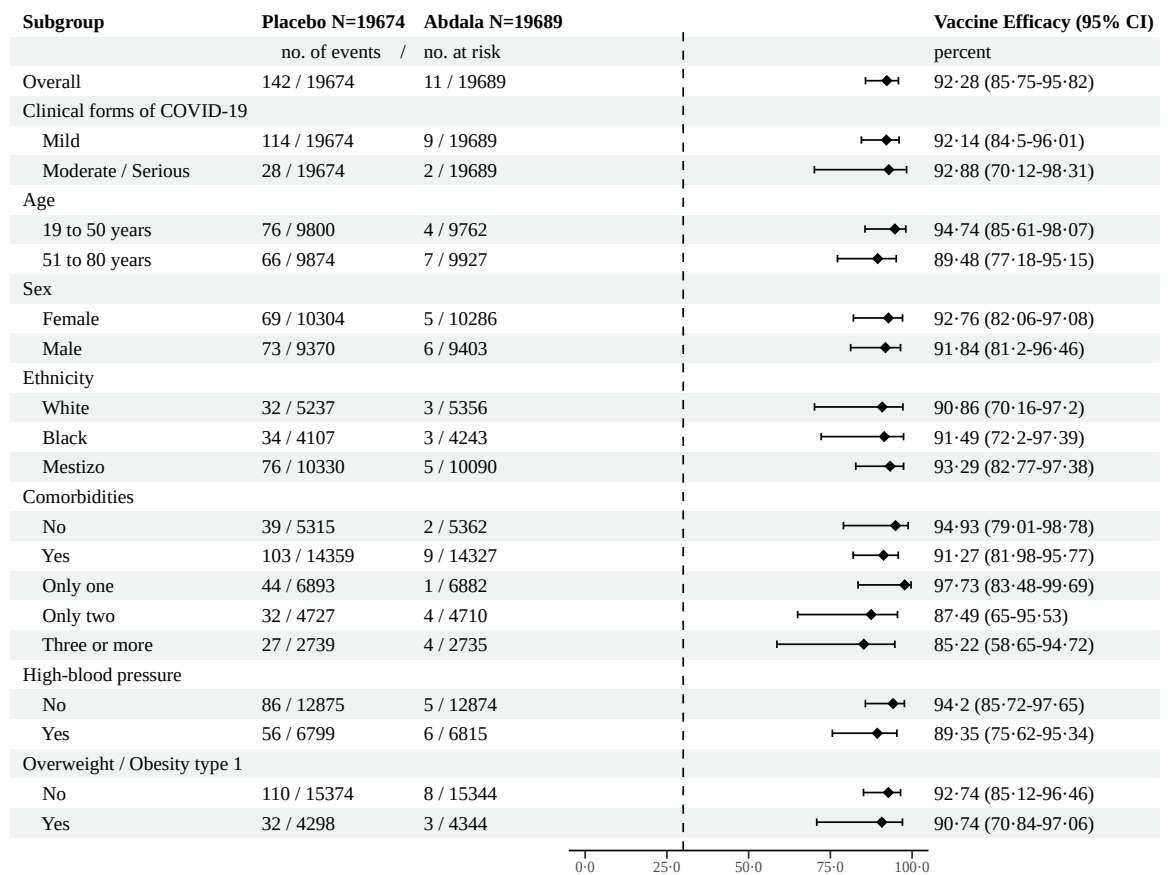
four deaths related to COVID-19, all in the placebo group.

The evaluation of the main efficacy outcomes occurred during May–June 2021, starting at May 3rd, in the context of high circulation of mutant viruses, predominantly variant of concern (VOC) Beta. In the PP population of 39,363 participants, 153 cases of virologically confirmed symptomatic COVID-19 with an onset at least 14 days after the third dose occurred: 142 in placebo recipients and 11 in Abdala recipients. This corresponded to a VE of 92.28% (95% CI 85.74–95.82) which was the primary end-point of the trial (Fig. 3).

Cumulative incidences of COVID-19 related events in Abdala and placebo groups are shown in Fig. 4. The case incidence per 1000 person-years (95% CI) was 78.44 (95% CI 66.07–92.46) for the placebo group and 6.05 (95% CI 3.02–10.82) for the Abdala group.

A secondary endpoint was efficacy evaluation according to COVID-19 clinical forms. In mild forms (114 cases in the placebo group and 9 in the Abdala group), VE was 92.14% (95% CI 84.50–96.01). Moderate/serious forms of COVID-19 (including critical disease) occurred in 30 participants (28 in the placebo group and only two in the Abdala group) for a VE of 92.88% (95% CI 70.12–98.31). In the placebo group, five critically ill patients as well as four deaths occurred, but none of those outcomes were found in Abdala recipients.

VE in preventing symptomatic COVID-19 was calculated in age subgroups of 19–50 and 51–80 years, as planned in the protocol (Fig. 3). In the age group of 19–50 years, VE was 94.74% (95% CI 85.61–98.07) and 89.48% (95% CI 77.18–95.15) for the group of 51–80 years. In the older group, 66 cases of COVID-19 occurred in the placebo group vs. only 7 in those who received Abdala. Interestingly, in the subset of the oldest

Subgroup	Placebo N=19674	Abdala N=19689		Vaccine Efficacy (95% CI)
	no. of events /	no. at risk		percent
Overall	142 / 19674	11 / 19689		92·28 (85·75-95·82)
Clinical forms of COVID-19				
Mild	114 / 19674	9 / 19689		92·14 (84·5-96·01)
Moderate / Serious	28 / 19674	2 / 19689		92·88 (70·12-98·31)
Age				
19 to 50 years	76 / 9800	4 / 9762		94·74 (85·61-98·07)
51 to 80 years	66 / 9874	7 / 9927		89·48 (77·18-95·15)
Sex				
Female	69 / 10304	5 / 10286		92·76 (82·06-97·08)
Male	73 / 9370	6 / 9403		91·84 (81·2-96·46)
Ethnicity				
White	32 / 5237	3 / 5356		90·86 (70·16-97·2)
Black	34 / 4107	3 / 4243		91·49 (72·2-97·39)
Mestizo	76 / 10330	5 / 10090		93·29 (82·77-97·38)
Comorbidities				
No	39 / 5315	2 / 5362		94·93 (79·01-98·78)
Yes	103 / 14359	9 / 14327		91·27 (81·98-95·77)
Only one	44 / 6893	1 / 6882		97·73 (83·48-99·69)
Only two	32 / 4727	4 / 4710		87·49 (65-95·53)
Three or more	27 / 2739	4 / 2735		85·22 (58·65-94·72)
High-blood pressure				
No	86 / 12875	5 / 12874		94·2 (85·72-97·65)
Yes	56 / 6799	6 / 6815		89·35 (75·62-95·34)
Overweight / Obesity type 1				
No	110 / 15374	8 / 15344		92·74 (85·12-96·46)
Yes	32 / 4298	3 / 4344		90·74 (70·84-97·06)

0·0   25·0   50·0   75·0   100·0

Fig. 3: **Abdala vaccine efficacy to prevent symptomatic COVID-19.** Secondary end-points and Subgroups analyses. Analysis of Abdala's efficacy in the prevention of symptomatic COVID-19 by clinical forms of the disease, death and various subgroups in the per-protocol population, was based on adjudicated assessments starting 14 days after the third injection. Vaccine efficacy, defined as 1 minus the hazard ratio (Abdala vs. placebo), and 95% confidence intervals were estimated with the use of a stratified Cox proportional-hazards model. The dashed vertical line represents a vaccine efficacy of 30%, based on the null hypothesis that the primary efficacy of Abdala vaccine is 30% or less.
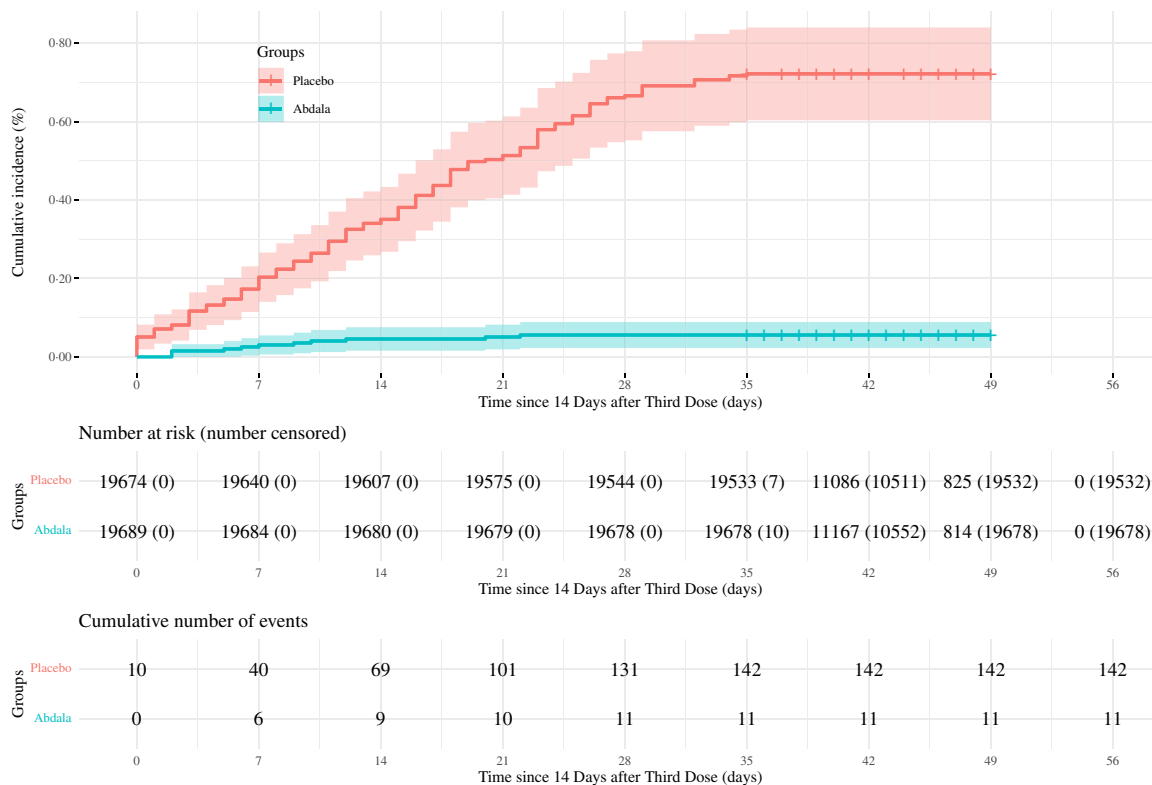
Fig. 4: Kaplan–Meier plots of efficacy of abdala vaccine against symptomatic COVID-19. Cumulative incidence of symptomatic COVID-19 in the per-protocol population is shown. The timing of surveillance for symptomatic COVID-19 participants began at least 14 days after the administration of the third dose (i.e., on day 42) through approximately the first 2 months of follow-up.

individuals (66–80 years), 19 out of 3735 from the placebo group had symptomatic COVID-19 but it did not occur in the 3670 individuals who received Abdala.

Additional analyses of VE in preventing COVID-19 were performed in subgroups of defined sex, and ethnicity, as well as the presence or absence of coexisting conditions (Fig. 3). VE according to sex and ethnicity was above 90%. In participants with/without comorbidities, the efficacy was 91.27% (95% CI 81.98–95.77) and 94.93% (95% CI 79.01–98.72), respectively. The point estimate of efficacy decreased as the number of comorbidities increased. Finally, the two most frequent comorbidities found in the trial (hypertension and overweight/obesity type 1) were analysed separately, showing VE estimates also above 90% (Fig. 3). Most of symptomatic cases in the efficacy analyses had some comorbidity (103/142 cases in the placebo group and 9/11 for the Abdala group). Incidence rate per 1000 person-years (95% CI) for all secondary variables is shown in Supplemental Material (S4, Table S4).

VE in preventing symptomatic COVID-19 in the mITT population was 83.29% (95% CI 76.55–88.1). Details showed in Supplemental Material (S5, Table S5, Fig. S1).

Discussion

A three-dose vaccination schedule of Abdala administered at 0-14-28 days showed high efficacy against symptomatic COVID-19 (92.28%), fulfilling the WHO target product profile for COVID-19 vaccines that suggests a "clear demonstration of efficacy (on a population basis) ideally with a point estimate around 50%" should be a minimum acceptable criterion for any COVID-19 vaccine, and as a preferred condition, at least 70% with consistent results in older individuals.[10] Both were achieved in this phase 3 trial. WHO recommends that successful vaccines should show an estimated hazard reduction of at least half, accurately enough to conclude that the true efficacy of the vaccine is greater than 30%.[10] It is estimated that a 50% effective vaccine could significantly reduce the incidence of COVID-19 among those vaccinated and could provide useful herd immunity. As a result, even if a much higher efficiency of 50% would be preferable, that would represent substantial progress.[11]

Worldwide, more than 360 vaccines against SARS-CoV-2 based on a broad range of technological platforms are currently under development, 170 of them already in clinical trials, including 43 in phase 3 and 11 vaccines have been included in the WHO's emergency

authorisation list.[12] Results of the efficacy of different vaccines are already published[13–20] but the comparison between them has been difficult considering the differences in study protocols, dose regimen, the evaluation in non-equivalent conditions in different countries and economic conditions, various stages of COVID-19 pandemic and SARS-CoV-2 variants, among other factors.[21–23]

Currently, according to the results of clinical trials, highly efficacious vaccines against symptomatic COVID-19 have been obtained[19]: mRNA-BNT162b2 vaccine 95% (95% CI 90.3–97.6), ChAdOx1 nCoV-19 (adenovirus vector vaccine) 70.4% (95% CI 95% 54.8–80.6), rAd26 and rAd5 vector-based (Gam-COV-IDVac) 91.6% (95% CI 85.6–95.2), Ad26.COV2.S (adenovirus vector vaccine) 66.1% (95% CI 55.0–74.8) and mRNA-1273 94.1% (95% CI 89.3–96.8). According to these results, Abdala's VE, shown in the phase 3 trial, compares favourably to all of this vaccines, produced using very different technology platforms.

Subunits vaccines also showed high efficacy levels against symptomatic COVID-19 in clinical trials[24]: Novavax/NVXCoV2373 (full length recombinant S protein-micelle nanoparticle/matrix M) 89.7% (95% CI 80.2–94.6), Soberana-02/Soberana-Plus (recombinant RBD conjugated to tetanus toxoid/RBD-dimer in Alum) 92.0% (95% CI 80.4–96.7),[25] SCB-2019 (recombinant trimeric spike protein/AS03 or CpG/Alum) 67.2% (95% CI.72% CI 54.3–76.8).[26] Our results show that Abdala's VE is very high, even though it is a simple vaccine formulation based on recombinant RBD in alum.

Consequently, regarding VE, Abdala behaves satisfactorily, as other vaccines widely use in worldwide mass immunization programs. Abdala has an excellent safety profile, previously observed in phase 1–2 clinical trial[5] and confirmed in the current phase 3 study. It was well tolerated, without any serious toxicity, and no deaths were attributed to its administration. In general, subunit vaccines have been very safe, in accordance with this technological platform.[24] One strength of Abdala is that the technology of gene expression of proteins in the yeast *P. pastoris* has more than thirty years-experience at CIGB.[27] Other key strength of Abdala is its high thermostability.[4] This offers a great advantage for the vaccination programs considering that no freezing conditions are required for storage and transportation, that makes this vaccine more affordable for countries or regions of difficult access, without conditions to maintain those freezing requirements.

Abdala's VE showed other encouraging results, such as those regarding with the efficacy against moderate/serious forms of COVID-19 (92.88%) as well as the fact that, the five critical patients found in the trial (of which four died) were all in the placebo group. VE according to age was also high, above 90%, consistent with the primary efficacy end-point. Considering that mortality from SARS-CoV-2 disproportionately affects older

adults, it is very important to develop vaccination strategies to protect this highly vulnerable subgroup. In the study, no symptomatic COVID-19 cases occurred in the oldest participants (66–80 years). An elevated proportion of participants had pre-existing conditions, such as hypertension and overweight type I obesity in accordance with the occurrence in real world settings in Cuba. When the absence or presence of any comorbidity was analysed, the efficacy values were again higher than 90%. The higher the number of comorbidities, the lower the point estimates of efficacy. Efficacy results regarding comorbidities are relevant, considering the influence of these control variables on the clinical outcome of COVID-19 patients, where severe forms of the disease and mortality in adults have been associated with the presence of pre-existing medical conditions.[28,29]

To our knowledge, Abdala's study is one of the largest phase 3 trials published on COVID-19 vaccines, enrolling more than 48,000 people in less than 14 days. The trial performed well, without deviations and a minimum of dropouts (1.4%). This was also facilitated by the compact immunization schedule of three doses administered at 0-14-28 days, previously selected in the phase 2 trial, very useful to face the urgency of the pandemic situation.[5] Randomisation assured a homogenous demographic and baseline characteristics of the participants in both placebo and Abdala groups. Blinding was maintained throughout the trial up to the estimation of clinical efficacy. Therefore, the internal validity of the study was adequate. Several factors such as the inclusion of participants from both sexes and different ethnic groups, the wide range of ages (19–80 years), the fact that 74% of the study population reported previous comorbidities/risk factors, as well as the high sample size of the trial, contribute to the generalisability (external validity, applicability) of the trial findings, narrowing the gap between the clinical trial and real-world populations, in which the same immunization schedule was used.

In Cuba, three epidemic periods have been defined as associated with the circulation of three major variants. Our phase 3 clinical trial was conducted from March to June 2021, corresponding exactly with the second period/second wave of the pandemic in the country,[30] characterized by a continuous increase in the incidence of cases associated to the expansion of the circulation of the Beta variant. Variant distribution changed over time, according to the emergence and expansion of those with the greatest evolutionary advantage. Abdala's vaccine was evaluated in the context of high circulation of mutant viruses in the country. According to the epidemiological data from the eastern province of Santiago de Cuba, that included most of the phase 3 volunteers, the huge increase in the number of COVID-19 cases in that period was explained by the predominant circulation of VOC Beta strain. Detailed information in Supplemental Material (S6).

An observational retrospective cohort study, carried out in Havana, analysed Cuba's Ministry of Public Health databases to estimate Abdala's real-world vaccine effectiveness.[31] The study, that included 1,355,638 persons age ≥19, demonstrated that Abdala was highly effective in preventing severe illness and death from COVID-19 (primary outcomes) under real-life conditions. In fully-vaccinated individuals, estimated Abdala's effectiveness was 98.2% (95% CI 97.9–98.5) against severe illness and 98.7% (95% CI 98.3–99.0) against death. Effectiveness exceeded 92.0% in all age groups. Those results, obtained in real-world conditions, complemented our findings obtained in the setting of the phase 3 placebo controlled, randomised, double blind clinical trial. The evaluation of Abdala's VE and effectiveness occurred in two separated geographically regions (eastern provinces and the western city of Havana, respectively) and two different epidemic waves.[30] As mentioned previously, Abdala's VE was evaluated during the second wave/second period of the pandemic characterized by the expansion of Beta variant. The evaluation of effectiveness occurred in the third transmission period, coinciding with the dissemination of the Delta variant. In Havana, the epidemic peak occurred in August, 2021, corresponding with the almost exclusive circulation of the Delta strain, considered the most transmissible variant globally at that time.

This study has several limitations. First, the short duration of the efficacy follow-up, as has previously occurred for many other vaccine candidates in Phase 3 trials. It is important to take into consideration that we carried out a unique inclusion strategy, where the 48,290 participants were included in less than 14 days, with a uniform immunization schedule of three doses in a 28 days period and a median time of follow-up since randomization up to efficacy database closure of 75 days (IQR 73, 79 days). On the other hand, as the incidence of SARS-CoV-2 increased in the country with the circulation of VOC, the time required to meet the efficacy end-point decreased. Second, this study did not assess the efficacy in other populations such as children, adolescents and pregnant and lactating women, but further studies in those populations were also conducted after EUA was granted. Third, in the current report, data regarding neutralizing antibody titters as well as the duration of the immune response were not included nor the demonstration of vaccine effectiveness in the current epidemic context, where different sub lineages of Omicron VOC are prevalent. The evaluation of all these issues is ongoing and we will be included in other publications.

To our knowledge, Abdala is the first COVID-19 vaccine based on a subunit RBD-protein obtained by recombinant-DNA technology in the yeast *P. pastoris,* which demonstrated clinical efficacy in phase 3 clinical trials. Abdala vaccine is safe and highly protective against symptomatic COVID-19, including the most severe forms of the disease and death. Those results, along with its immunization schedule and the advantage of easy storage and handling conditions at 2–8 °C, make this vaccine an option for the use in immunization strategies as a key tool for the control of the pandemic.

Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2023.100497.

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