Safety and efficacy of the two doses conjugated protein-based SOBERANA-02 COVID-19 vaccine and of a heterologous three-dose combination with SOBERANA-Plus: a double-blind, randomised, placebo-controlled phase 3 clinical trial

María Eugenia Toledo-Romaní,[a,s,*] Mayra García-Carmenate,[b,s] Carmen Valenzuela-Silva,[c,s] Waldemar Baldoquín-Rodríguez,[a] Marisel Martínez-Pérez,[d] Meiby Rodríguez-González,[d] Beatriz Paredes-Moreno,[d] Ivis Mendoza-Hernández,[e] Raúl González-Mujica Romero,[d] Oscar Samón-Tabio,[f] Pablo Velazco-Villares,[g] Juan Pablo Bacallao-Castillo,[g] Ernesto Licea-Martín,[d] Misladys Rodríguez-Ortega,[a] Nuris Herrera-Marrero,[a] Esperanza Caballero-González,[d] Liudmila Egües-Torres,[b] Reinaldo Duartes-González,[h] Serguey García-Blanco,[i] Suzette Pérez-Cabrera,[j] Santos Huete-Ferreira,[k] Kirenia Idalmis-Cisnero,[l] Omayda Fonte-Galindo,[m] Dania Meliá-Pérez,[n] Ivonne Rojas-Remedios,[o] Delaram Doroud,[p] Mohammad Mehdi Gouya,[q] Alireza Biglari,[p] Sonsire Fernández-Castillo,[d] Yanet Climent-Ruiz,[d] Yury Valdes-Balbín,[d,s] Dagmar García-Rivera,[d,s] Patrick Van der Stuyft,[r] and Vicente Verez-Bencomo,[d,s,**] the SOBERANA Phase 3 team[t]

[a]"Pedro Kourí" Tropical Medicine Institute. Av "Novia del Mediodía", Kv 6 1/2, La Lisa, Habana, 11400, Cuba
[b]Havana Hygiene and Epidemiology Center, Havana, Cuba
[c]Cybernetics, Mathematics and Physics Institute. 15th St #55, Vedado, Plaza de la Revolución, Havana, 10400, Cuba
[d]Finlay Vaccine Institute. 21st Ave. N° 19810 between 198 and 200 St, Atabey, Playa, Havana, Cuba
[e]National Coordinating Center for Clinical Trials. 5th Ave and 62, Miramar, Playa, Havana, Cuba
[f]"José Antonio Echevarría" Technological University, Havana, Cuba
[g]UCT Geocuba Research and Consulting, Havana, Cuba
[h]Municipal Health Department, Centro Habana, Havana, Cuba
[i]Municipal Health Department, La Lisa, Havana, Cuba
[j]Municipal Health Department, 10 de Octubre, Havana, Cuba
[k]Municipal Health Department, Habana Vieja, Havana, Cuba
[l]Municipal Health Department, Cerro, Havana, Cuba
[m]Municipal Health Department, Marianao, Havana, Cuba
[n]Municipal Health Department, Playa, Havana, Cuba
[o]Municipal Health Department, Plaza Havana, Cuba
[p]Pasteur Institute of Iran. No. 69, Pasteur Ave., Tehran 1316943551, Islamic Republic of Iran
[q]Iran University of Medical Science, Teheran, Iran
[r]Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

Summary

Background SOBERANA-02 is a COVID-19 conjugate vaccine (recombinant RBD conjugated to tetanus toxoid). Phases 1/2 clinical trials demonstrated high immunogenicity, promoting neutralising IgG and specific T-cell response. A third heterologous dose of SOBERANA-Plus (RBD-dimer) further increased neutralising antibodies. The aim of this study is to evaluate the safety and efficacy of two immunisation regimes: two doses of SOBERANA-02 and a heterologous three-dose combination with SOBERANA-Plus added to it.

Methods From March 8th to June 24th, 2021 we conducted in Havana, Cuba a multicentre randomised, double-blind, placebo-controlled, phase-3 trial evaluating a two doses SOBERANA-02 scheme and a heterologous scheme with one dose SOBERANA-Plus added to it (RPCEC00000354). Participants 19–80 years were randomly assigned to receiving 28 days apart either the two or three dose scheme or placebo. The main endpoint was vaccine efficacy in preventing the occurrence of RT-PCR confirmed symptomatic COVID-19 at least 14 days after the second or third dose in the per-protocol population. We also assessed efficacy against severe disease and, in all participants receiving at least one vaccine/placebo dose, safety for 28 days after each dose.

The Lancet Regional Health - Americas 2023;18: 100423

Published Online 31 December 2022
https://doi.org/10.1016/j.lana.2022.100423

*Corresponding author.
**Corresponding author.
E-mail addresses: mariaeugenia@ipk.sld.cu (M.E. Toledo-Romaní), vicente.verez@finlay.edu.cu (V. Verez-Bencomo).
[s]Contributed equally to this article.
[t]The members of the SOBERANA Phase 3 team are listed in the Supplementary information (S1 and S2).

Findings We included 44,031 participants (52.0% female, 48.0% male; median age 50 years, range 19–80 years; 7.0% black, 24.0% mixed-race, 59.0% white) in a context of initial Beta VOC predominance, with this variant being partially replaced by Delta near the trial's end. Vaccine efficacy in the heterologous combination was 92.0% (95%CI 80.4–96.7) against symptomatic disease. There were no severe COVID-19 cases in the vaccine group against 6 in the placebo group. Two doses of SOBERANA-02 was 69.7% (95%CI 56.5–78.9) and 74.9% (95%CI 33.7–90.5) efficacious against symptomatic and severe COVID-19, respectively. The occurrence of serious and severe adverse events (AE) was very rare and equally distributed between placebo and vaccine groups. Solicited AEs were slightly more frequent in the vaccine group but predominantly local and mostly mild and transient.

Interpretation Our results indicate that the straightforward to manufacture SOBERANA vaccines are efficacious in a context of Beta and Delta VOC circulation, have a favourable safety profile, and may represent an attractive option for use in COVID-19 vaccination programmes.

Funding This study received funds from the National Fund for Science and Technology (FONCI-CITMA-Cuba, contract 2020–20) of the Ministry of Science, Technology and Environment of Cuba.

Keywords: Conjugate-vaccine; SARS-COV-2; COVID-19 vaccine; Heterologous scheme; RBD-subunit vaccine; Vaccine efficacy

Research in context

Evidence before this study

We searched PubMed for research articles and reviews published until July 1st, 2022, using the terms "efficacy" AND "subunit COVID-19 vaccines" without further filters or restrictions. We found 143 papers, one of them a living systematic review that pools estimates of vaccine efficacy against symptomatic COVID-19 for different platforms and reports: mRNA vaccines 95% (95%CI 92–97); protein subunit vaccines 77% (95%CI 5–95); viral vector vaccines 68% (95%CI 61–74); inactivated vaccines 61% (95%CI 52–68). By restricting the search to "Clinical trial" [Publication Type]) AND "heterologous vaccination" [MeSH Terms] AND "COVID-19 vaccine" [MeSH Terms] we retrieved only 6 papers, with just one trial related to heterologous combinations for priming immunisation versus COVID-19.

We have previously reported safety and immunogenicity results of phase 1/2 clinical trials with 2 doses of SOBERANA-02 (FINLAY-FR-2), the first conjugate vaccine developed for SARS CoV-2 immunisation, and with a three-dose heterologous scheme adding SOBERANA-Plus (FINLAY-FR-1A) to it. The 2 doses regimen induced neutralizing antibodies and T-cells with IFNγ secretion, indicative of a Th1 pattern. After the third heterologous dose, which significantly increased neutralizing anti-RBD IgG titres, IgG antibodies were detected against D614G and VOCs alpha, Beta, Delta and Omicron and were still present 7–8 months after the third dose.

Added value of this study

We report on a multicentre, phase 3 randomised, placebo-controlled, double-blind trial conducted in Havana, to evaluate the efficacy and safety of vaccination against SARS-CoV-2. The trial included three groups receiving 28 days apart either two doses of SOBERANA-02, or a heterologous scheme with a dose of SOBERANA-Plus added to it, or placebo. It was conducted in a context of initial predominance of Beta VOC, with this variant being partially replaced by Delta towards the end of the observation period. The vaccine efficacy (VE) of the two doses scheme was 69.7% (95%CI 56.5–78.9) 14 days after the second dose administration. VE for the heterologous three-dose combination was 92.0% (95%CI 80.4–96.7). Severe COVID-19 occurred in 23 and 5 participants in the placebo and SOBERANA-02 group (VE 74.9%; 95%CI: 33.7–90.5) and led to 3 and 2 deaths, respectively. Fourteen days after the third dose, severe disease occurred in 6 and 0 placebo and SOBERANA-Plus group participants, respectively; no death was observed in any of the groups. The occurrence of serious and severe AEs was infrequent and similarly distributed between the placebo and vaccine groups. Solicited AEs were slightly more frequent in the vaccine group but predominantly local and mostly mild and transient.

Implications of all the available evidence

SOBERANA-02, the first protein based conjugate vaccine against SARS CoV-2, and SOBERANA-Plus are straightforward to manufacture and have logistical advantages in terms of storage and distribution, due to being stable and permitting the use of a conventional 2–8 °C cold-chain. The vaccines are efficacious in a context of Beta and Delta VOC circulation, have a favourable safety profile, and constitute an attractive, feasible option for resource constrained low- and middle-income settings.

Introduction

Immunisation with an effective vaccine constitutes the main tool to fight the COVID-19 pandemic, and achieving high vaccination coverage is critical to control the emergence and spread of new SARS CoV-2 variants.[1] Clinical trials provided evidences of the efficacy of vaccines based on different technologies, which received WHO endorsed emergency use authorisation.[2] Notwithstanding, even under optimistic manufacturing and delivery scenarios, global equitable access to vaccines is likely to be limited when relying on the currently available products only.[3]

SOBERANA-02 is a subunit conjugated protein-based COVID-19 vaccine. The antigen is the recombinant receptor binding domain (RBD) protein conjugated chemically to tetanus toxoid (TT).[4] The well-known protein-polysaccharide conjugation technology provided novel, safe and highly immunogenic paediatric vaccines against *Haemophilus influenzae* type b from the 1980s onwards,[5] and for protecting against *Neisseria meningitidis* and *Streptococcus pneumoniae*,[6,7] among others.

In phase 1 and 2a clinical trials two doses of SOBERANA-02 induced neutralising antibodies and T-cells with IFNγ secretion, indicative of a Th1 response pattern.[8] A single dose of SOBERANA-Plus (RBD-dimer/Alum) demonstrated booster capacity in convalescent COVID-19 patients who had acquired natural immunity against the virus,[9] through hybrid immunity.[10] A third heterologous dose of SOBERANA-Plus after two doses of SOBERANA-02 significantly increased neutralising anti-RBD IgG titres.[8] These results were confirmed in a phase 2b clinical trial.[11]

To determine whether the immunogenicity results would translate into differentiated clinical efficacy outcomes we set up a phase 3 trial (IFV/COR/09 number, RPCEC00000354) with a placebo control arm that evaluated the safety and the efficacy of two immunisation regimes: two doses of SOBERANA-02, and the heterologous combination thereof with SOBERANA-Plus as a third dose.

Methods

Study design and context

A multicentre, adaptive, randomised, placebo-controlled, double-blind phase 3 trial was set up to evaluate the efficacy and safety of vaccination against SARS-CoV-2 with 2 doses of SOBERANA-02 (FINLAY-FR-2) -subsequently referred to as So2-and with a three dose heterologous scheme adding SOBERANA-Plus (FINLAY-FR-1A) to the former -hereafter So2P. Participants were recruited from March 8th to March 31st, 2021 and received, with intervals of 28 days, either the two or three doses scheme or placebo.

The study was conducted in Havana, Cuba. Comprehensive primary health care services with a defined population of responsibility are a key element of the Cuban health care system. They consist of family doctor/nurse practices and policlinics. The practices are the systems' entry point, policlinics provide diagnostic and support services and more specialized care. The routine identification of COVID-19 cases is based on these first line services, which pinpoint suspects and refer to hospitals. Upon a positive RT-PCR test they are admitted until symptom resolution with a negative PCR test. Contacts of COVID-19 cases are isolated for 5 days and routinely PCR tested.

The epidemiological context at the start of the trial and during So2 efficacy evaluation coincided with the second epidemic wave in Havana (S4. Fig. S1; data sources: Havana Hygiene and Epidemiology Center, and Virology Department, "Pedro Kourí" Tropical Medicine Institute, Ministry of Public Health).[12] At the start of the So2 efficacy evaluation, VOC Beta predominated (epidemiological week 16–20: 83.6% to 72.7%). Later, after initiating the So2P efficacy evaluation, the Beta strain was partially replaced by Delta (epidemiological week 20–26: 72.7% to 47.3% Beta and 2.2% to 52.6% Delta, respectively). The latter was associated with a sharp rise of transmission leading to a significant increase in the incidence rate of clinical cases from 119.4 to 172.7 × 10^5 in epidemiological weeks 20 and 26, respectively.

Roles and responsibilities

The trial was sponsored by the Finlay Vaccine Institute. A central Research Ethics Committee was appointed *ad hoc* by the Cuban Ministry of Public Health. It approved the protocol (Clinical Trials IFV/COR/09 number, RPCEC00000354) and the informed consent forms. In the event of a medical problem requiring unmasking, approval by the principal investigator was planned for. All principles of the Helsinki Declaration and the International Council for Harmonization guidelines were adhered to.[13] Full details on procedures are provided in the protocol.

The National Clinical Trials Coordinating Centre (CENCEC) monitored the trial for protocol adherence and observance of Good Clinical Practice and oversaw data accuracy. An Independent Safety Data Monitoring Board (ISDMB) continuously supervised safety and conducted interim analysis. The ISDMB had access to the case files, confirmed severe cases of COVID-19 illness, and assessed whether any deaths were SARS CoV-2-related.

Participants, randomisation and blinding

In 8 municipalities of west Havana we set up, in existing health infrastructure, 48 vaccination and clinical sites. Practitioners of the family practices linked to each of these sites, organised information campaigns in the community and summoned the population between 19

and 80 years old to participate in the study. The family doctors carried out a first eligibility screening before transferring potential participants to the nearest vaccination site, where the medical doctors decided the inclusion.

Subjects between 19 and 80 years old with no known history of SARS-CoV-2 infection that consented to use reliable anticonception if female and in fertile age, and that provided written informed consent for participation were included. Exclusion criteria were previous receipt of a COVID-19 vaccine, acute febrile illness or infectious disease, pregnancy, puerperium or breastfeeding, any uncontrolled non-communicable disease, history of a severe allergic reaction to any component of the vaccines, current or planned (within 30 days) receipt of immunomodulatory drugs, history of COVID-19 infection, decreased mental ability to take decisions, tattoos in the deltoid region of both arms, and administration of tetanus anatoxin-containing vaccines within the last 3 months. At inclusion, a blood sample for asserting rapid serologic evidence of SARSCoV-2 prior asymptomatic infection (Realy Tech IgG/IgM, China) was collected in order to subsequently permit safety and efficacy analyses stratified by prior exposure.

Block randomisation into the placebo, So2 and So2P study arms and placebo was done at a 1:1:1 ratio, stratified by site and by a priori defined risk strata: 19–64 years without risk of severe COVID-19, 19–64 years with risk of severe disease, and ≥65 years. The size of the blocks by site and risk strata was adjusted in each case to the potential of subjects to include in each stratum, with a minimum of 33 and a maximum of 159. Participants younger than 65 years of age were categorised as being at risk of severe COVID-19 if they had at least one of the following self-referred risk factors: obesity (body mass index ≥35), severe malnutrition (BMI < 18.5), hypertension (according to the Ministry of Public Health guidelines),[14] chronic kidney disease, ischemic heart disease, diabetes mellitus, chronic obstructive pulmonary disease, asthma (grade 2/3), cancer, HIV or primary/secondary immunodeficiency. The research product management specialist generated the random allocation sequence. Each participant got an identification code, which matched the vial label code.

The presentation of the vaccine candidates and placebo were undistinguishable. The participants, the involved health care workers and the investigators were masked to group allocation until the decision was taken to administer or complete the three-dose vaccine schedule.

Products under evaluation

SOBERANA-02 is a subunit protein-based vaccine. The antigen is the recombinant SARS-CoV-2 RBD (25 μg), chemically conjugated to tetanus toxoid and adsorbed on 500 μg alumina.[4,8] SOBERANA-Plus antigen is a dimeric RBD (50 μg) adsorbed on 1250 μg alumina.[15,16] The placebo contained SOBERANA-02 ingredients except the active principle. All were stored at 2–8 °C and distributed daily to the clinical sites.

Efficacy assessment

The primary endpoint was vaccine efficacy (VE) for preventing occurrence of symptomatic COVID-19 infection, confirmed by a positive SARS-CoV-2 RT-PCR nasopharyngeal swab (RT-PCR), with onset at least 14 days after the last injection in the per-protocol population (PPP). Secondary endpoints were the efficacy for the prevention of severe COVID-19 and death attributed to COVID-19. Agreement with case definition criteria for primary and secondary endpoints was judged blindly by COVID-19 hospital doctors and reviewed by specifically trained medical research team members. The study's Principal Investigator resolved any discrepancy. COVID-19 symptomatic disease was considered if participants had at least one major symptom or sign (dyspnoea, oxygen saturation ≤92%, persistent thoracic pain, neurological disorders, clinical or radiographic evidence of pneumonia) or two minor symptoms (fever, chills, myalgia, headache, sore throat, running nose, diarrhoea/vomiting, myalgia, malaise, cough, dysgeusia/anosmia). In that case RT-PCR (QIAGEN, Germany) confirmation was performed by the SARS CoV-2 national reference laboratory at the "Pedro Kourí" Tropical Medical Institute, Havana, Cuba.

Severe COVID-19 was defined by the presence of any of the following: dyspnoea with respiratory rate >30 breaths/min; cyanosis, pulmonary infiltration/condensation, oxygen saturation ≤90% or assisted mechanical ventilation, acute respiratory distress syndrome, evidence of septic shock, or admission to intensive care. Death was attributed to COVID-19 if the cause could be attributed to a complication of COVID-19.

Safety assessment

All adverse events (AE) were recorded in face-to-face medical consultations on the three consecutive days after receiving each dose of vaccine or placebo and by self-report using a diary of adverse events up to 28 days. AEs were classified as solicited (within 7 days after injection) and unsolicited (within 28 days after injection); local and systemic; grade 1, 2 or 3 (according to the Common Terminology Criteria for Adverse Events, version 5.0),[17] and causally vaccine related or not (during 28 days) following WHO recommendation.[18]

Data storage and management

All information generated during the study was recorded on hard copy forms specifically designed for the trial. We utilized the "OpenClinic" medical record system (http://openclinic.sourceforge.net) to electronically

store study subjects' general demographic and clinical information, specific data on the possible occurrence and subsequent course of a symptomatic COVID-19 disease episode, and information on adverse events. We made use of the national online registry ANDARIEGO.HIGIA (GEOCUBA, Cuba, http://higia.andariego.cu) platform to store the participants' vaccination records and follow up their PCR results. The platform registers COVID-19 doses administered in all vaccination centres in Cuba, and results of PCR tests performed in all laboratories of the health system.

To digitalise the primary information, we employed data operators who were trained in the use of the software packages. Both platforms provide an exhaustive change control record, ensuring security and integrity, and allow data export. A unique code assigned to each subject at enrolment guaranteed confidentiality and permitted linking information from both sources. The sponsor's staff ensured data export and cleansing for the subsequent carrying out the interim and final analyses. All data management activities were governed by standardised working procedures.

We developed a monitoring and audit plan for quality control. During site visits, the proper conduct of the trial was verified by assessing adherence to the study protocol, good clinical practise, and standard operating procedures. We systematically checked the completion of all records and the quality of digitalization. The visits also made it possible to detect and correct any differential assessments of clinical events between centres.

Statistical analysis (see also S3- Supplemental statistical methods)

In line with international recommendations that the primary efficacy endpoint estimate for a placebo-controlled trial should be at least 50% to ensure that a widely deployed COVID-19 vaccine is effective,[19] the trial was designed to test the hypothesis that the risk of symptomatic COVID-19 is reduced >60% compared to placebo group. With uniform recruitment and assuming a six month cumulative incidence of 0.66% symptomatic cases in the placebo group, the design required inclusion of 44,010 subjects (14,670 per study group) and a total of 151 events (between the Placebo group and each vaccination scheme) to achieve 90% power (and 2.5% 1-sided type I error) to detect a hazard ratio (HR) of 0.4 (VE >60% to reduce the risk of symptomatic disease compared to placebo), with a null hypothesis of HR 0.7. VE bounds were derived using a Lan-DeMets O'Brien-Fleming approximation spending function. Two interim analyses (IA) were planned at detection of 53 and 106 symptomatic cases meeting the primary outcome definition.

We used person-time of exposure in the summary statistics and group comparisons. Vaccine efficacy was calculated in the per-protocol population (PPP) as percentage reduction in the HR: $VE = 100 \times (1 - HR)$ %, in the vaccine groups as compared to the placebo group, with HR estimated from a stratified Cox proportional hazards model taking into account the defined risk strata. To take the evolution of the epidemiological situation over time into account, the VE for So2P was estimated considering the So2P and control arm cases occurring in the same calendar period (14 days after dose 3 in So2P and 42 days after the 2nd dose in placebo).

Safety was assessed in all participants in the safety population (those who received at least one injection). Descriptive summary statistics (numbers and percentages) for participants with any solicited AE, unsolicited AE, unsolicited severe AE, serious AE, and AE leading to discontinuation from the trial are provided by arm and after each dose.

A criterion for stopping due to unacceptable toxicity (frequency of serious vaccine related adverse event >1%) was evaluated iteratively with a Bayesian algorithm (see study protocol).

Statistical analyses were performed using R version 4.2.0.[20]

Decision to stop the trial

Considering the advantageous results demonstrated in the interim analyses, and the explosive epidemiological situation, it was deemed unethical to withhold the benefits of the vaccine from individuals in the placebo group. The Central Research Ethics Committee of the Ministry of Public Health recommend the sponsor to start offering to participants randomised in that group the three-dose schedule. As a consequence, the observation period for efficacy estimation of So2P versus placebo was limited to 6 weeks. Concurrently, So2 participants were invited to receive a third heterologous dose of SOBERANA-Plus.

Role of the funding source

The funder had no role in data collection, data analysis, data interpretation, or writing of the manuscript.

Results

Trial population

Between March 8–31st, 2021, 45.184 volunteers were screened and 44,031 were randomly assigned to So2 (14.679), So2P (14,677) or placebo (14,675) (Fig. 1). Participants' characteristics were balanced between the study arms (Table 1) and the gender and racial distribution were representative of the Cuban demographic structure (see also S4-Table S3 for characteristics by age and risk of severe COVID-19). At baseline, serologic evidence of a previous SARS-CoV-2 infection was detected in 0.3% of participants. All participants

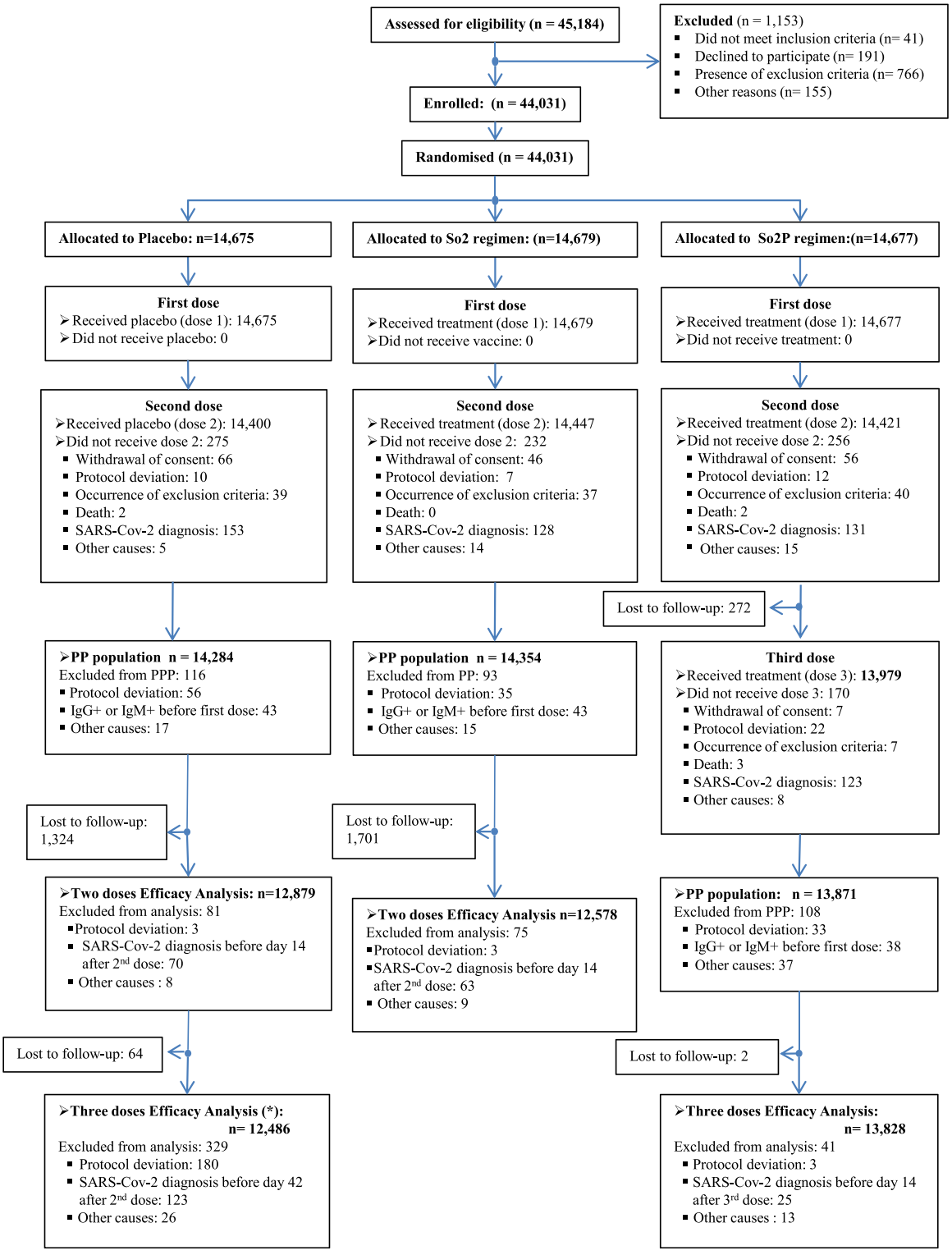

***Fig. 1:* Study flow chart.** So2 regimen: two doses of SOBERANA-02, every 28 days; So2P regimen: heterologous combination of two doses of SOBERANA-02 and a third dose with SOBERANA-Plus, every 28 days. *Considering cases occurring in the same calendar period for the comparison with three dose schedule (42 days after the 2nd dose of placebo) Protocol deviations (364) were due to intercurrent vaccination with another product (53.8%), errors in the administration of the vaccine formulation (11.2%), incorrect inclusion (2.7%) and other reasons (32.1%).

Characteristic	Treatment[b]			
	Overall N = 44,031	Placebo N = 14,675	So2 N = 14,679	So2P N = 14,677
Age–Median (range) - years	50 (19–80)	50 (19–80)	50 (19–80)	50 (19–80)
Race or ethnic group - n (%)				
Black	7276 (17.0)	2453 (17.0)	2379 (16.0)	2444 (17.0)
Mixed-race	10,738 (24.0)	3574 (24.0)	3575 (24.0)	3589 (24.0)
White	26,017 (59.0)	8648 (59.0)	8725 (59.0)	8644 (59.0)
Sex - n(%)				
F	23,102 (52.0)	7662 (52.0)	7694 (52.0)	7746 (53.0)
M	20,929 (48.0)	7013 (48.0)	6985 (48.0)	6931 (47.0)
Comorbidities - n (%)	18,074 (41.0)	5968 (41.0)	6064 (41.0)	6042 (41.0)
Asthma	2926 (6.6)	994 (6.8)	945 (6.4)	987 (6.7)
COPD	311 (0.7)	95 (0.6)	104 (0.7)	112 (0.8)
Cancer	263 (0.6)	96 (0.7)	79 (0.5)	88 (0.6)
Obesity	1772 (4.0)	592 (4.0)	577 (3.9)	603 (4.1)
Cardiovascular disease	1336 (3.0)	454 (3.1)	430 (2.9)	452 (3.1)
Diabetes mellitus	3673 (8.3)	1194 (8.1)	1224 (8.3)	1255 (8.6)
CKD	111 (0.3)	40 (0.3)	36 (0.2)	35 (0.2)
Immunodeficiency	2636 (6.0)	870 (5.9)	872 (5.9)	894 (6.1)
HBP	13,703 (31)	4529 (31)	4572 (31)	4602 (31)
Severe malnutrition	354 (0.8)	120 (0.8)	128 (0.9)	106 (0.7)
Age category and risk for severe Covid-19[a] - n (%)				
≥65 years	7908 (18.0)	2634 (18.0)	2639 (18.0)	2635 (18.0)
19–64 years	23,642 (54.0)	7895 (54.0)	7875 (54.0)	7872 (54.0)
19–64 years with any comorbidity	12,481 (28.0)	4146 (28.0)	4165 (28.0)	4170 (28.0)
Baseline RT-PCR test - n (%)				
Negative	44,009 (99.9)	14,666 (99.9)	14,676 (99.9)	14,667 (99.9)
Positive	22 (<0.1)	9 (<0.1)	3 (<0.1)	10 (<0.1)
Baseline IgM or IgG anti-SARS-CoV-2 rapid test - n (%)				
Negative	43,905 (99.7)	14,632 (99.7)	14,635 (99.7)	14,638 (99.7)
Positive	126 (0.3)	43 (0.3)	44 (0.3)	39 (0.3)

[a]Participants are at risk of severe COVID-19 with at least one of the following comorbidities: obesity (body mass index ≥35), severe malnutrition (BMI < 18.5), hypertension, chronic kidney disease, ischemic heart disease, diabetes mellitus, chronic obstructive pulmonary disease, asthma (grade 2/3), cancer, HIV or primary/secondary immunodeficiency. [b]Treatment: So2: Two doses of SOBERANA-02, 28 days apart; So2P: Two doses of SOBERANA-02 and 1 dose of SOBERANA-Plus, 28 days apart.

Table 1: **Baseline Demographic and Clinical Characteristics of the randomized participants.**

received the first dose of the treatment they were allocated to.

After receiving the first dose, 168 (0,38%) participants withdrew their consent and did not receive the second dose and after that dose, 7 (0,05%) participants from the So2P group withdrew their consent. 412 (0.94%) and 123 (0.84%) participants diagnosed with symptomatic PCR positive SARS-CoV-2 infection were excluded before receiving the second and the third dose, respectively. Protocol deviations - 364 (0.83%) in total-were due to intercurrent vaccination with another vaccine (53.8%), errors in the administration of the candidate product (11.2%), incorrect inclusion (2.7%) and other reasons (32.1%). The PPP primary efficacy analysis included 42509 subjects: 14354 in the So2, 13871 in the So2P, and 14284 in the placebo group. Follow-up from 14 days after the second dose in the two doses analysis totalled 70,959 and 64,926 persons-weeks in the placebo and So2 arm, respectively, while in the three doses analysis the corresponding follow-up time was 20,047 persons-weeks in the placebo and 47,088 persons-weeks in the So2P group.

Safety

Local solicited Vaccine-Associated Adverse Events (VAAEs) occurred somewhat more frequently in the vaccine group than in the placebo group after the first dose (7.7%, vs. 2.6%, 95%CI for difference: 4.7–5.5%) and the second dose (1.9%, vs. 0.4%, 95%CI for difference: 1.3–1.7%) (Fig. 2, S6-Table S4, S7-Table S5). After the third dose, the frequency of subjects with local AEs dropped to 0.3% (S7-Table S6). In the vaccine groups 97.5% of local adverse events were grade 1 and less than 0.2% grade 3. They lasted a median of 2 days after the first and second dose (25–75th percentile: 1–3 after the first and 1–2 after the 2nd dose), and 2 days after the third dose (25–75th percentile: 0–3). The most common local AE was pain at the injection site (2.6%, 7.5% and

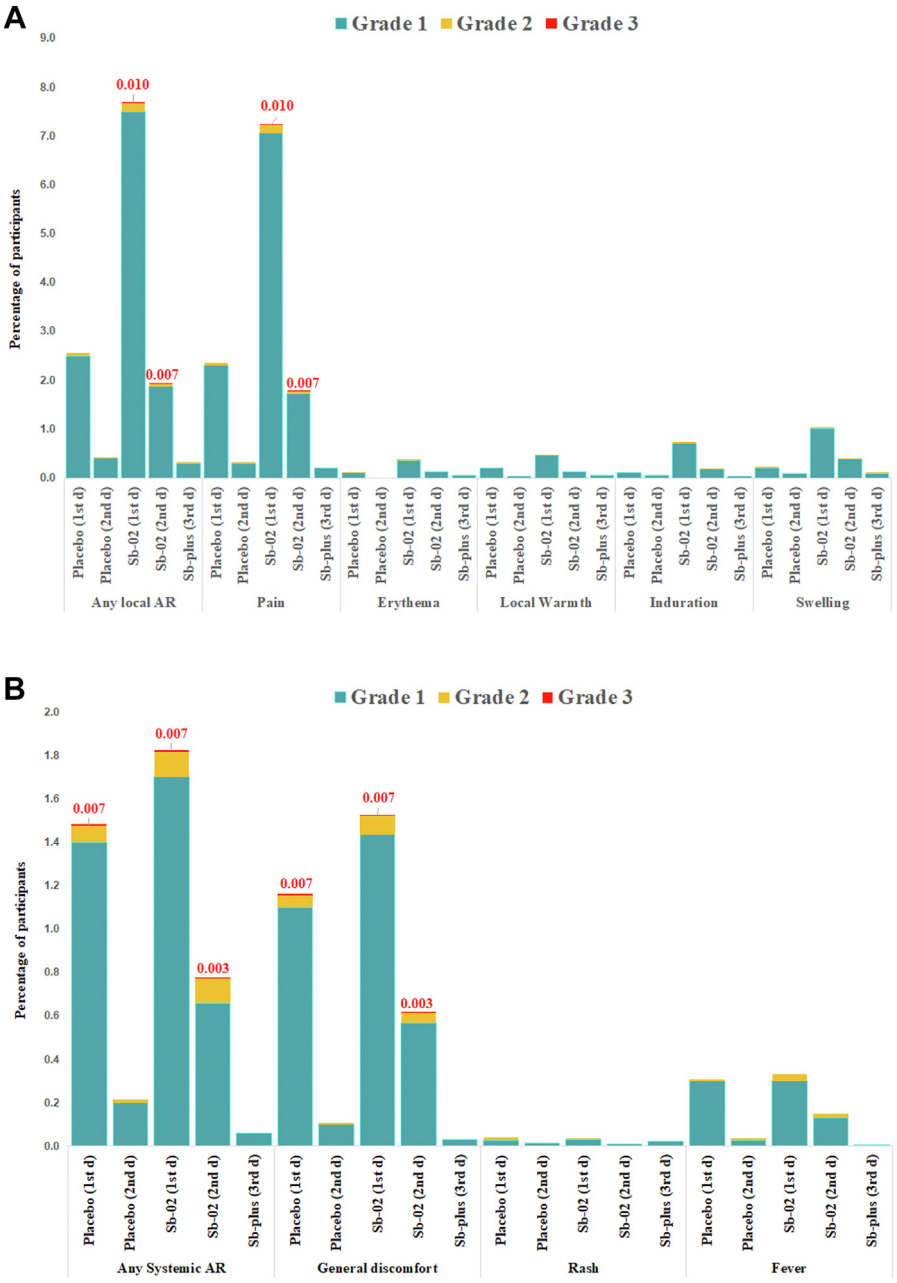

Fig. 2: **Solicited Vaccine-Associated Adverse Events (VAAE) within 7 days after each dose by grade.** A) Local AEs; B) Systemic AEs. Numbers in red above the bars indicate the % grade 3 AE.

8.7% in placebo and vaccine groups, respectively). Onset of injection–site reactions on or after day 8 were noted in 21 participants (0.05%) after the first dose (4 for placebo and 17 for vaccine groups), in 4 (0.01%) after the second dose (2 for placebo and 2 for vaccine groups) and in 1 participant (0.01%) after the third dose. Local reactions were characterized by erythema, induration, warmth, swelling and pain, and resolved after a median of 1 day (25–75th percentiles: 0.5–2).

Solicited systemic VAAEs occurred a little more often in the vaccine groups than in the placebo group after both the first dose (1.8% vs. 1.4%; 95%CI for difference: 0.1–0.6%) and the second dose (0.7% vs. 0.2%; 95%CI for difference: 0.4–0.6%) and dropped to 0.1%

after the third dose (Fig. 2, S6-Table S4, S7-Table S5, S8-Table S6). Their severity in the vaccine groups changed slightly from 6.5% after the first dose to 8.8% after the second dose for grade 2 events, and from 0.6% to 0.4% for grade 3 ones. Solicited systemic AEs in the vaccine groups lasted a median of 1 day after the first (25–75th percentile: 0–2) and second doses (25–75th percentile: 0–3), and 2 days after the third dose (25–75th percentile: 0.8–7.5). The frequency of solicited AE was very low in subject with serologic evidence of a past SARSCoV-2 infection at intake: 0.1% of local AE after any injection and <0.1% of systemic events.

Unsolicited AE frequency reported during the 28 days following each injection was similar in all groups. The more frequent events (≥1%) were a high blood pressure measurement, mainly in subjects with known hypertension (1.8% for placebo, 2.1% for So2 and 2.2% for So2P) and headache (1.0% for placebo, 1.2% for So2 and 1.5% for So2P) (S9-Table S8). The occurrence of serious (<0.1%) and severe (0.1%) VAAE was equal between groups (S8-Table S7, S10-Table S9). 3.9% of all the AEs were grade 3 in the placebo group against 3.4% and 4.2% for groups So2 and So2P, respectively and 7.9%, 6.7% and 6.7% were serious in these groups, respectively. All-cause mortality was 24 (0.2%) amongst placebo recipients against 9 and 11 (0.1%) in groups So2 and So2P (P = 0.014). No death was vaccine related.

Efficacy

The final analysis of the two-doses schedule included 174 cases of symptomatic COVID-19: 136 in the placebo group (99.9 per 1000 person-years; 95%CI 83.8–118.2) and 38 in the So2 group (30.5 per 1000 person-years; 95%CI 21.6–41.9). This corresponds to 69.7% VE (95% CI 56.5–78.9%; P < 0.001) for the prevention of symptomatic SARS-CoV-2 infection by the So2 schedule (Table 2, Fig. 3, S13-Fig. S2). The vaccine efficacy to prevent COVID-19 was congruous across subgroups stratified by demographic and baseline characteristics (S14-Fig. S3).

The VE for prevention of severe COVID-19 (23 cases in placebo vs 5 in So2) was 74.9% (95%CI: 33.7–90.5) (S12-Table S10). There were 3 and 2 deaths from COVID-19 disease, respectively; the small number of events precludes a point estimate of VE.

For the final analysis of the three-dose heterologous So2P schedule 41 cases of COVID-19 were included: 35 in the placebo group (91.4 per 1000 person-years; 95% CI 63.4–126.6) and 6 in the So2P group, (6.6 per 1000 person-years; 95%CI 2.4–14.5). This means 92.0% VE (95%CI 80.4–96.7%; P < 0.001) for prevention of symptomatic SARS-CoV-2 infection by the 3-dose schedule (Table 2, Fig. 3, S15-Fig. S4). Subgroup's analysis yields remarkably consistent VE estimates, but lacks precision for much of the strata (S16-Fig. S5). Severe COVID-19 occurred in 6 and 0 participants in the placebo and So2P group, respectively (S12-Table S10). There were no deaths from COVID-19 disease.

Including in the analysis subjects that were PCR negative but had a IgG/IgM positive test at inclusion, indicating past SARSCoV-2 infection, did not affect any of the VE estimates reported in all the above PPP analysis.

Discussion

The SOBERANA vaccines demonstrated to be efficacious in preventing PCR-confirmed symptomatic SARS-CoV-2 infections among adults aged 19–80 years. Efficacy after two doses SOBERANA-02 administration, 69.7%, was well over the WHO-set 50% threshold.[19] After completion of the heterologous schedule with SOBERANA-Plus, VE attained an excellent 92.0%. Efficacy to prevent severe COVID-19 was 74.9% for the two doses regimen; no severe case presented under the heterologous scheme. The occurrence of serious and severe AEs was very rare

COVID-19 disease	Two-dose Vaccine Efficacy	
	Placebo	So2
n of events, PPP	136	38
Person- weeks follow-up	70,959	64,926
Incidence rate per 1000 Person-Years (95% CI)	99.9 (83.8; 118.2)	30.5 (21.6; 41.9)
Vaccine Efficacy (95%CI)		69.7 (56.5; 78.9)
	Heterologous three-dose Vaccine Efficacy	
	Placebo	So2P
n of events, PPP	35	6
Person- weeks follow-up	20,047	47,088
Incidence rate per 1000 Person-Years (95% CI)	91.4 (63.4; 126.6)	6.6 (2.4; 14.5)
Vaccine Efficacy (95% CI)		92.0 (80.4; 96.7)

Vaccine efficacy was defined as (1 - minus the hazard ratio)*100%, (Vaccine vs. placebo), and the 95% confidence interval was estimated with the use of a stratified Cox proportional hazards model.

Table 2: **Vaccine Efficacy to prevent symptomatic COVID-19 disease in the Per-Protocol Population (PPP).**

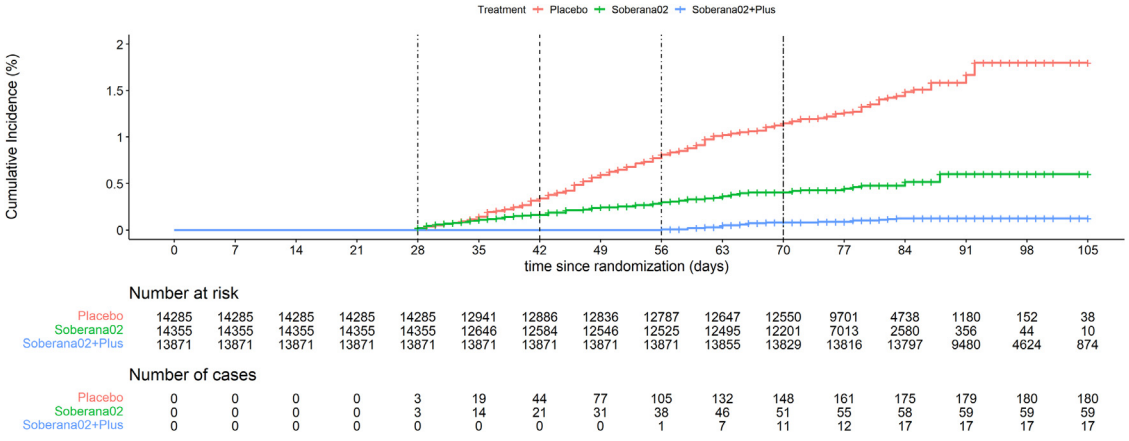

***Fig. 3*: Cumulative incidence of COVID-19 disease.** Vertical dashed lines indicate the start of dose 2 administration, So2 efficacy evaluation, dose 3 administration and So2P efficacy evaluation, respectively.

and equally distributed between the placebo and vaccine groups. Solicited AEs were slightly more frequent in the vaccine group but predominantly local and mostly mild and transient.

Subunit protein vaccines present clear advantages in terms of manufacturing, storage and distribution.[21] Still, administration of a third heterologous dose in the So2P regimen, which significantly further increases SOBERANA's VE, could be construed as a disadvantage compared to COVID-19 vaccines using two dose regimens. However, the need for and benefits of administering a booster dose to the primary schedules of registered vaccines is increasingly recognised.[22,23] Furthermore, due to the T-epitopes from the tetanus toxoid carrier protein, SOBERANA-02 -the first conjugate SARS CoV-2 RBD protein-based vaccine induces T-helper response and long lasting immunity.[8,11] Heterologous combinations for priming and booster using protein vaccines are also reported by others, with excellent results.[24,25]

Our preclinical results highlighted the stimulation of specific B- and T-memory cells, affinity maturation and a significant and boostable IgG immune response in mice.[4] The immunogenicity pattern observed in phase 1 and 2a trials suggested as best option two doses of 25-µg SOBERANA-02 followed by a third dose of SOBERANA-Plus.[8] In phase 2b the 4-fold anti-RBD IgG seroconversion rate in 758 volunteers was 76.3% after two doses and 96.8% after the third heterologous dose, against 7.3% in the placebo group.[11] Neutralising IgG antibodies were detected against D614G and VOCs Alpha, Beta, Delta and Omicron, and neutralising antibodies were still present 7–8 months after the third dose.[11] The results of the present trial permit to conclude that the immunogenicity findings translate into improved clinical efficacy of the 3-dose scheme.

A living systematic review reports pooled vaccine efficacy against symptomatic COVID 19 for different platform as: mRNA vaccines 95% (95%CI: 92%–97%); protein subunit vaccines 77% (95%CI 5%–95%); viral vector vaccines 68% (95%CI: 61%–74%) inactivated vaccines 61% (95%CI 52%–68%]. It found that viral vector vaccines decreased overall mortality (risk ratio = 0.25; 95%CI 0.09–0.67), but analogous estimates for other platform vaccines are imprecise.[26] SOBERANA's performance in the present study compares favourably with the above findings.

Furthermore, the trial was conducted in a challenging epidemiological context: VOC Beta was by far predominant during So2 efficacy evaluation and partially replaced by VOC Delta after starting So2P evaluation. Beta has demonstrated significant immune evasion ability. It carries the E484K mutation that substantially reduces neutralisation by antibodies in sera of convalescent and vaccinated persons.[27] The few available VE trials on Beta, most of them conducted in South Africa, show efficacy declines with respect to the performance under predominance of the original SARS-CoV-2 strain, except for BNT162b2 (Pfizer/BioNTech), with reported efficacy of 100% (95%CI 53.5–100.0%).[28] ChAdOx1-nCoV-19 (AstraZeneca) was not efficacious against mild-to-moderate Beta variant disease,[29] a single dose Ad26.COV2.S (Janssen) 52.0% (95% CI 30.3–67.4%) against moderate disease,[30] and NVX-CoV2373 (Novavax) 60.1% (95%CI 19.9–80.1) against symptomatic disease.[31]

Two doses of SOBERANA-02 achieved 69.7% (95% CI 56.5–78.9%), a quite remarkable efficacy against VOC Beta in our racially diverse Latin-American trial population. One could surmise that, being a conjugated protein-based vaccine that triggers a broad immune response, the efficacy might remain relatively well at par

against other immune evading variants. The heterologous combination, 92.0% efficacious, performed still better. It is, however, not possible to fully unravel the efficacy with a third SOBERANA-Plus dose from the partial replacement of Beta by Delta VOC. It should also be noted that, in line with previous phase 3 COVID-19 vaccine trials, we did not assess effects against asymptomatic infection.

Notwithstanding, both variants have evasion ability and observational studies have been providing evidence of reduced effectiveness of WHO authorised emergency use listing vaccines against both.[2] A systematic review and metanalysis of real-world effectiveness of COVID-19 vaccines against Delta VOC showed consistently lower protection against symptomatic disease than found in the earlier efficacy studies with the original strain. After a full vaccination course with 2 doses, the meta-analysis effectiveness was 83.7% (Pfizer/BioNTech) and 77.5% (Moderna) for mRNA-based vaccines, and 80.1% for the AstraZeneca vector vaccine.[32]

The encouraging safety profile observed during the phase 2 SOBERANA clinical trial was confirmed in this phase 3 study.[8,11] Pain at the injection site was not uncommon, akin to what has been observed for other subunit protein and mRNA COVID 19 vaccines,[23] but mostly mild, while rates of other local reactions were low. Fever and general discomfort were rather uncommon and generally mild, as were other systemic reactions. Serious and severe AEs occurred very rarely and similarly in the vaccine and placebo groups and no death was vaccine related, but sample sizes far beyond those of a typical clinical trial may be needed to adequately appraise these frequencies.

The Central Research Ethics Committee had deliberated that it was not acceptable to administer 56 days after randomisation a placebo dose in the So2 and placebo arms -coincident with the SOBERANA-Plus third dose in the So2P group- and demanded to remove administration of this placebo dose from the study protocol. Conceptually, the So2P group was "unblinded" and the So2P-placebo VE estimate may have been subjected to some behaviour driven bias. However, participants in the placebo comparison group remained blinded to their actual allocation to either 2 doses of placebo or 2 doses of SOBERANA-02, and repeated standardised instructions for all study participant on what constitute signs and symptoms of COVID-19 infection and how to proceed, mass information campaigns encouraging care seeking when suspecting infection, contact tracing and screening in the population at large, and general enforcement of protective measures such as mask wearing and social distancing, should have minimised such bias, if any.

The decision to stop the trial early and to offer full 3 dose vaccination to all participants due ethical concerns constitutes, from a methodological point of view, a limitation of our study. It shortened especially the follow up period for assessing the efficacy of the three dose So2P schedule. Still, to observe waning of vaccine protection at least 6 months follow up would be needed, which can generally only be achieved in observational studies under real life conditions. However, because of that decision, Placebo and So2 group participants had to be censored in the PPP analysis when they received a first and a third vaccine dose, respectively. This did not markedly affect the precision of our estimates but performing an ITT analysis was ruled out. Instead of possibly mimicking more closely the effectiveness of the vaccine in routine practice, such an analysis would in this case result in fatally biasing the VE estimates downward, below the "real world" effectiveness -and to the greatest extent so for the 3 doses VE, where the proportion of "contaminated by vaccination" person-time in the placebo group would be exceedingly high. Therefore, we only report PPP results, allowing for comparison with other trial publications, which generally emphasize PPP efficacy anyway.

Recruitment of participants through family medicine practices, which guaranteed accelerated inclusion reflecting the composition of the population in terms of gender, race and pre-existing conditions, constitutes a strength of this study. Coordination with the health system's established and well adhered to routine SARS-CoV-2/COVID-19 activities for case ascertainment, hospitalization and standardised case management also contribute to the strengths.

Recombinant protein COVID-19 vaccines are stable and can be stocked at 2° to 8 °C in a regular refrigerator and are easier to distribute and use than those vaccines that must be stored at much lower temperatures and require powerful freezers. This represents a key advantage in less-wealthy countries. Furthermore, they rely on an established and widely used vaccine technology, and their production can be cost-effectively implemented and scaled up in biotechnology facilities in the global south that are manufacturing other protein subunit products. Overall, our results indicate that the SOBERANA vaccines are efficacious in a context of Beta and Delta VOC circulation, have a favourable safety profile, and constitute an attractive, feasible option for use in COVID-19 vaccination.

Contributors

METR, CVS, YVB, DGR and VVB conceptualised the study. METR, MGC, ECG, LET, RDG, SGB, SPC, SHF, KIC, OFG, DMP, IRR, MRO, NHM were clinical investigators. MRG, BPM, IMH supervised and monitored the trial. CVS, WBR and PVdS performed the statistical analysis. RGMR, OST, PVV, JPBC and ELM were involved in data acquisition and data curation. SFC, YCR, YVB, MMP were responsible for vaccine development, manufacturing and project administration. DD, MMG and AB contributed to trial design. METR, CVS, WBR, DGR, YVB, VVB and PVdS wrote the manuscript. All authors critically reviewed the manuscript for important intellectual content and approved the final version. METR, MGC and CVS directly accessed and verified the underlying data. VVB and METR were responsible for the decision to submit the paper.

The members of SOBERANA Phase 3 team participated in different processes during the trial: adverse event classification, data collection, treatment of adverse events, treatment of COVID-19 cases, quality assurance, and on site coordination and monitoring of the trial, among others.

Data sharing statement

The protocol (English version) is available upon submission. Summary data on adverse events and VE are shared in the Supplementary Material. Information is also available at the Cuban Public Registry of Clinical Trials, included in WHO International Clinical Trials Registry Platform (https://rpcec.sld.cu/en/trials/RPCEC00000354-En). Other supporting clinical documents, including the statistical analysis plan and the informed consent form will be made available after publication. Proposals for full data sharing should be sent to: mariaeugenia@ipk.sld.cu, vicente.verez@finlay.edu.cu or cifinlay@finlay.edu.cu. These proposals will be reviewed and must be approved by the sponsor and the senior investigators. Lastly, a data access agreement must be signed.

Declaration of interests

VVB, YVB, DGR, YCR, SFC are authors of two patent applications. The other authors declare no competing interests. No author received an honorarium for contributing to this paper.

Acknowledgments

We especially thank all the volunteers who participated in the trial and the members of the SOBERANA-Phase 3 clinical trial team (listed in the Supplementary Information) for their dedication and contributions to the trial.

We further thank the IDSMB members: N. A. Jimenez Perez (Chair and Clinical Expert) and C. M. Fonseca Gómez (Clinical Expert) from the Pedro Kourí Tropical Medicine Institute, G. María Suárez Formigo (Immunological Expert) and P. Lorenzo-Luaces Alvarez (Statistical Expert) from the Center for Molecular Immunology, M. Martínez Cabrera (Clinical Expert) from the Ministry of Public Health.

We also thank Rolando Pérez, Ileana Morales and Eduardo Martínez (BioCubaFarma), and Lila Castellanos for their advice and support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2022.100423.

References

1 Krause PR, Fleming TR, Longini IM, et al. SARS-CoV-2 variants and vaccines. *N Engl J Med*. 2021;385:179–186. https://doi.org/10.1056/NEJMsr2105280.
2 Rotshild V, Hirsh-Raccah B, Miskin I, et al. Comparing the clinical efficacy of COVID-19 vaccines: a systematic review and network meta-analysis. *Sci Rep*. 2021;11:22777. https://doi.org/10.1038/s41598-021-02321-z.
3 WHO. Vaccine equity. https://www.who.int/campaigns/vaccine-equity. Accessed February 22, 2021.
4 Valdes-Balbin Y, Santana-Medero D, Quintero L, et al. SARS-CoV-2 RBD-tetanus toxoid conjugate vaccine induces a strong neutralising immunity in preclinical studies. *ACS Chem Biol*. 2021;16:1223–1233. https://doi.org/10.1101/2021.02.08.430146.
5 Schneerson R, Robbins JB, Barrera O, et al. Haemophilus influenzae type B polysaccharide-protein conjugates: model for a new generation of capsular polysaccharide vaccines. *Prog Clin Biol Res*. 1980;47:77–94.
6 Goldblatt D. The indirect effect of pneumococcal conjugate vaccine. *Lancet Glob Health*. 2017;5:e6–e7. https://doi.org/10.1016/S2214-109X(16)30338-2.
7 Gasparini R, Panatto D. Meningococcal glycoconjugate vaccines. *Hum Vaccin*. 2011;7:170–182. https://doi.org/10.4161/hv.7.2.13717.
8 Toledo-Romani ME, Verdecia-Sánchez L, Rodríguez-González M, et al. Safety and immunogenicity of anti-SARS CoV-2 vaccine SOBERANA 02 in homologous or heterologous scheme: open label phase I and phase IIa clinical trials. *Vaccine*. 2022;40:4220–4230. https://doi.org/10.1016/j.vaccine.2022.05.082.
9 Chang-Monteagudo A, Ochoa-Azze R, Climent-Ruiz Y, et al. A single dose of SARS-CoV-2 FINLAY-FR-1A vaccine enhances neutralization response in COVID-19 convalescents, with a very good safety profile: an open-label phase 1 clinical trial. *Lancet Reg Health Am*. 2021;4:100079. https://doi.org/10.1016/j.lana.2021.100079.
10 Bates TA, Mcbride SK, Leier HC, et al. Vaccination before or after SARS-CoV-2 infection leads to robust humoral response and antibodies that effectively neutralize variants. *Sci Immunol*. 2022;7:eabn8014. https://doi.org/10.1126/sciimmunol.abn801.
11 Toledo-Romani ME, García-Carmenate M, Verdecia-Sánchez L, et al. Safety and immunogenicity of anti-SARS-CoV-2 heterologous scheme with SOBERANA 02 and SOBERANA Plus vaccines: phase IIb clinical trial in adults. *Med*. 2022;3:760–773. https://doi.org/10.1016/j.medj.2022.08.0011.
12 Guzmán MG, Perez L, Tejero Y, et al. Emergence and evolution of SARS-CoV-2 genetic variants during the Cuban epidemic. *J Clin Virol*. 2022;2:100104. https://doi.org/10.1016/j.jcvp.2022.100104.
13 Food and Drug Administration. E6(R2) good clinical practice: integrated addendum to ICH E6(R1). Guidance for Industry https://www.fda.gov/regulatory-information/search-fda-guidance-documents/e6r2-good-clinical-practice-integrated-addendum-ich-e6r1; 2018. Accessed February 2021.
14 Pérez-Caballero MD, León-Álvarez JL, Dueñas-Herrera A, et al. Cuban guide for hypertension diagnosis, evaluation and treatment. *Rev Cubana Med*. 2017;56:242–321. Available: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0034-75232017000400001&lng=es.
15 Ochoa-Azze R, Chang-Monteagudo A, Climent-Ruiz Y, et al. Safety and immunogenicity of the FINLAY-FR-1A vaccine in COVID-19 convalescent participants: an open-label phase 2a and double-blind, randomised, placebo-controlled, phase 2b, seamless, clinical trial. *Lancet Respir Med*. 2022;10:785–795. https://doi.org/10.1016/S2213-2600(22)00100-X.
16 Pérez-Rodríguez S, Rodríguez-González MC, Ochoa-Azze R, et al. A randomised, double-blind phase I clinical trial of two recombinant dimeric RBD COVID-19 vaccine candidates: safety, reactogenicity and immunogenicity. *Vaccine*. 2022;40:2068–2075. https://doi.org/10.1016/j.vaccine.2022.02.029.
17 US Department of Health and Human Services. *Common Terminology Criteria for Adverse Events (CTCAE). Version 5.0*. Washington: HHS; 2017. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50. Accessed February 2021.
18 World Health Organization. *Causality Assessment of an Adverse Event Following ImmunisationImmunisation (AEFI)*. 2nd ed. Geneva: WHO; 2018. https://appswho.int/iris/bitstream/handle/10665/259959/9789241513654-eng.pdf. Accessed December 10, 2020.
19 WHO consideration for an evaluation of COVID19 Vaccines. *Point to consider for manufacturers of COVID-19 vaccines*; 2020. https://www.who.int/default-source/COVID19. Accessed February 2021.
20 R Core Team. R: a language and environment for statistical computing. Available from: https://www.R-project.org/; 2022.
21 Pollet J, Chen WH, Strych U. Recombinant protein vaccines, a proven approach against coronavirus pandemics. *Adv Drug Deliv Rev*. 2021;170:71–82. https://doi.org/10.1016/j.addr.2021.01.001.
22 Ying W, Pak-Hang P. Effectiveness of heterologous and homologous covid-19 vaccine regimens: living systematic review with network meta-analysis. *BMJ*. 2022;377:e069989. https://doi.org/10.1136/bmj-2022-069989.
23 Shaw R, Stuart A, Greenland M, Liu X, Nguyen JVT, Snape M, Com-COV Study Group. Heterologous prime-boost COVID-19 vaccination: initial reactogenicity data. *Lancet*. 2021;397:2043–2046. https://doi.org/10.1016/S0140-6736(21)01115-6.
24 Stuart A, Shaw R, Liu X, et al. Immunogenicity, safety, and reactogenicity of heterologous COVID-19 primary vaccination incorporating mRNA, viral-vector, and protein-adjuvant vaccines in the UK (Com-COV2): a single-blind, randomised, phase 2, non-inferiority trial. *Lancet*. 2022;399:36–49. https://doi.org/10.1016/S0140-6736(21)02718-5.
25 Jin P, Guo X, Chen W, et al. Safety and immunogenicity of heterologous boost immunisation with an adenovirus type-5-vectored

and protein-subunit-based COVID-19 vaccine (Convidecia/ZF2001): a randomised, observer-blinded, placebo-controlled trial. *PLoS Med*. 2022;19:e1003953. https://doi.org/10.1371/journal.pmed.1003953.
26 Korang SK, von Rohden E, Veroniki AA, et al. Vaccines to prevent COVID-19: a living systematic review with Trial Sequential Analysis and network meta-analysis of randomised clinical trials. *PLoS One*. 2022;17(1):1–23. https://doi.org/10.1371/journal.pone.0260733.
27 Jangra S, Ye C, Rathnasinghe R, et al. SARS-CoV-2 spike E484K mutation reduces antibody neutralization. *Lancet Microbe*. 2021;2:283–284. https://doi.org/10.1016/S2666-5247(21)00068-9.
28 Thomas SJ, Moreira ED, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine through 6 months. *N Engl J Med*. 2021;385:1761–1773. https://doi.org/10.1056/NEJMoa2110345.
29 Madhi SA, Baillie V, Cutland CL, et al. Efficacy of the ChAdOx1 nCoV-19 covid-19 vaccine against the B.1.351 variant. *N Engl J Med*. 2021;384:1885–1898. https://doi.org/10.1056/NEJMoa2102214.
30 Sadoff J, Gray G, Vandebosch A. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. *N Engl J Med*. 2021;384:2187–2201. https://doi.org/10.1056/NEJMoa2101544.
31 Shinde V, Bhikha S, Hoosain Z, et al. Efficacy of NVX-CoV2373 Covid-19 vaccine against the B.1.351 variant. *N Engl J Med*. 2021;384:1899–1909doi. https://doi.org/10.1056/NEJMoa210305.
32 Pormohammad A, Zarei M, Ghorbani S, et al. Effectiveness of COVID-19 vaccines against Delta (B.1.617.2) variant: a systematic review and meta-analysis of clinical studies. *Vaccines*. 2022;10:23. https://doi.org/10.3390/vaccines10010023.