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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



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ABSTRACT

Background: SOBERANA 02 is a COVID-19 vaccine based on SARS-CoV-2 recombinant RBD conjugated to tetanus toxoid (TT). SOBERANA Plus antigen is dimeric-RBD. Here we report safety and immunogenicity from phase I and IIa clinical trials using two-doses of SOBERANA 02 and three-doses (homologous) or heterologous (with SOBERANA Plus) protocols.

Method: We performed an open-label, sequential and adaptive phase I to evaluate safety and explore the immunogenicity of SOBERANA 02 in two formulations (15 or 25 µg RBD-conjugated to 20 µg of TT) in 40 subjects, 19–59-years-old. Phase IIa was open-label including 100 volunteers 19–80-years, receiving two doses of SOBERANA 02–25 µg. In both trials, half of volunteers were selected to receive a third dose of the corresponding SOBERANA 02 and half received a heterologous dose of SOBERANA Plus. Primary outcome was safety. The secondary outcome was immunogenicity evaluated by anti-RBD IgG ELISA, molecular neutralization of RBD:hACE2 interaction, live-virus-neutralization and specific T-cells response.

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Results: The most frequent adverse event (AE) was local pain, other AEs had frequencies $\leq 5\%$. No serious related-AEs were reported. Phase IIa confirmed the safety in 60 to 80-years-old subjects.

In phase-I SOBERANA 02–25 μg elicited higher immune response than SOBERANA 02–15 μg and progressed to phase IIa. Phase IIa results confirmed the immunogenicity of SOBERANA 02–25 μg even in 60–80-years. Two doses of SOBERANA02-25 μg elicited an immune response similar to that of the Cuban Convalescent Serum Panel and it was higher after the homologous and heterologous third doses. The heterologous scheme showed a higher immunological response. Anti-RBD IgG neutralized the delta variant in molecular assay, with a 2.5-fold reduction compared to D614G neutralization.

Conclusions: SOBERANA 02 was safe and immunogenic in persons aged 19–80 years, eliciting neutralizing antibodies and specific T-cell response. Highest immune responses were obtained in the heterologous three doses protocol.

Trial registry: <https://rpcec.sld.cu/trials/RPCEC00000340>, <https://rpcec.sld.cu/trials/RPCEC00000347>

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1. Introduction

Safe and effective vaccines are urgently needed to globally control the spread of COVID-19 [1,2]. Novel vaccines based on mRNA and adenovirus-vector platforms [3–8] and more traditional vaccines—as whole inactivated virus or protein subunit vaccines— [9–12] have fulfilled the required efficacy threshold ($\geq 50\%$) [2] and received emergency use authorizations; however, $<5\%$ of doses administered worldwide have gone to low-income countries [13,14]. More than 100 COVID-19 vaccines are under clinical evaluation [15] and their success would be essential for reducing inequity in vaccine distribution worldwide. Among them, vaccines based on SARS-CoV-2 protein subunits have shown significant advantages concerning safety and conservation conditions, becoming more affordable for low- and middle-income countries [16].

SOBERANA 02 is a protein subunit conjugate vaccine in which RBD is conjugated to tetanus toxoid (TT). This is the only anti-SARS-CoV-2 conjugate vaccine in the clinical pipeline of WHO [15]; it is supported by a vast experience at Finlay Vaccine Institute on carbohydrate-protein conjugate vaccines [17,18]. By conjugating RBD to TT, both humoral and cellular immune responses are potentiated as the conjugate exposes multiple RBM (receptor binding motif) where neutralizing epitopes predominate [19]. In laboratory animals, RBD-TT elicited a robust neutralizing antibody response, a Th1-polarized T-cell response and immune memory [20].

SOBERANA 02 started phase I [21] (October 30th, 2020) and phase IIa [22] (December 17th, 2020) clinical trials for evaluating safety and immunogenicity in a two-doses scheme, followed by a third dose of SOBERANA 02 or SOBERANA Plus. SOBERANA Plus has been successfully evaluated as booster for COVID-19 convalescents [23,24]; here it is evaluated for the first time as third dose in a heterologous immunization scheme.

2. Methods

2.1. Products under evaluation

SOBERANA 02 and SOBERANA Plus are suspensions for injection. Both are subunit vaccines based on SARS-CoV-2 RBD, sequence Arg319-Phe541-(His)₆ bearing a flexible C-terminal fragment that includes unpaired Cys538, produced in genetically modified CHO cells. In SOBERANA 02, 15 or 25 μg of RBD are conjugated to 20 μg of the carrier protein tetanus toxoid (TT). In SOBERANA Plus-50 μg , the RBD is dimerized (d-RBD) through a Cys538–Cys538 interchain disulfide bridge. Both vaccines use aluminium hydroxide as adjuvant. SOBERANA 02 and SOBERANA Plus (Table 1) are produced under GMP conditions at the Finlay Vaccine Institute (IFV) and the Centre for Molecular Immunology (CIM), in Havana, Cuba.

2.2. Participants and study design

Eligible participants were healthy persons according to clinical and laboratory criteria, aged 19–59 years (phase I) or 19–80 years (phase IIa) of both sexes, recruited through public advertisement at community or professional environment close to the clinical site (Clinic #1, La Lisa Municipality in Havana). The health condition was assessed during the screening visit, based on medical records, physical examination, and clinical and microbiological laboratory tests. Key exclusion criteria were history of SARS CoV-2 infection, acute diseases, congenital or acquired immune system disease, personal history of liver or kidney failure, immunological treatment in the last three months, allergy to ingredients in the formulation, pregnancy, puerperium or breastfeeding (Supplemental Material, Appendix A-1 y A-2).

Table 1
Composition of vaccine candidates.

Ingredient	Vaccine candidates	
	SOBERANA 02	SOBERANA Plus
Antigen	SARS-CoV-2 RBD conjugated to tetanus toxoid, 15 μg or 25 μg RBD per 20 μg tetanus toxoid	SARS-CoV-2 RBD dimer (d-RBD), 50 μg
Aluminium hydroxide (alum)	0.5 mg	1.25 mg
Sodium chloride	4.25 mg	4.25 mg
Disodium hydrogen phosphate	0.03 mg	0.03 mg
Sodium dihydrogen phosphate	0.02 mg	0.02 mg
Water for injection	0.5 ml	0.5 ml

Phase I clinical trial was open-label, monocentric, sequential and adaptive to evaluate the safety and reactogenicity and to explore immunogenicity of two formulations of SOBERANA 02 of 15 or 25 µg. Forty volunteers were sequentially assigned to two groups. The 20 subjects assigned to arm 1 received the first dose of SOBERANA 02–15 µg (low dose formulation) and after the first interim analysis of safety on day 7, the subjects assigned to arm 2 received the first dose of SOBERANA 02–25 µg. On day 28, 39 subjects received the correspond second dose (one withdrew by voluntary abandonment on arm 2). On day 56 and after 6 withdrew (reasons declared in Fig. 1); half of volunteers in each arm were randomly allocated for receiving a third dose of SOBERANA 02 (homologous group, same formulation of the first immunizations) or SOBERANA Plus (50 µg of d-RBD/alumina, heterologous group) (Fig. 1).

Phase II was an adaptive clinical trial for evaluating immunogenicity, safety and reactogenicity of SOBERANA 02–25 µg. It was designed in two stages (IIa and IIb). Phase IIa started after the interim analyses from phase I (safety and preliminary immunogenicity after 1st dose), where SOBERANA 02–25 µg was selected. It was open-label and included 100 volunteers aged 19–80 years (19–59: 76 subjects, 60–80: 24 subjects), receiving two doses of SOBERANA 02–25 µg on days 0 and 28. On day 56, participants were randomly allocated to receive either a third dose of SOBERANA 02–25 µg or SOBERANA Plus (Fig. 2). Phase IIb included 810 volunteers in a double blind, randomized, placebo-controlled trial and will be published separately.

Both trials are published in the Cuban Public Registry of Clinical Trials, included in WHO International Clinical Registry Trials Platform with codes RPCEC00000340 and RPCEC00000347 [21,22].

2.3. Ethical considerations

Phase I clinical trial was approved by the Ethical Committee at the Cuban National Centre for Toxicology. Phase IIa was approved by a Research Ethic Committee from the Medical Sciences University, Faculty of Medicine “Manuel Fajardo”, Havana, designed by the Health Innovation Committee from the Cuban Ministry of Health (MINSAP) for clinical trials of COVID-19 vaccines. The Cuban National Regulatory Agency (Centre for State Control of Medicines and Medical Devices, CECMED) approved the trials and the procedures (CECMED, Authorizations dates: 29th October 2020 for phase I, Reference number: 05.014.20BA, and 17th December for phase II, Reference number: 05.019.20BA).

The National Clinical Trials Coordinating Center (CENCEC) was responsible for monitoring the trial in terms of adherence to the protocol, Good Clinical Practice and data accuracy.

Both trials were conducted according to Helsinki’s Declaration, Good Clinical Practice and the Cuban National Immunization Program. During recruitment, the investigators provided the potential participants with oral and written information about the vaccine candidates and trial potential risks and benefits. Written informed consent was obtained from all participants. The decision to participate was voluntary and was not remunerated.

2.4. Procedures

Participants received intramuscular injections in the deltoid region. After each vaccination, they were closely followed for safety evaluation (during three hours in phase I and one hour in

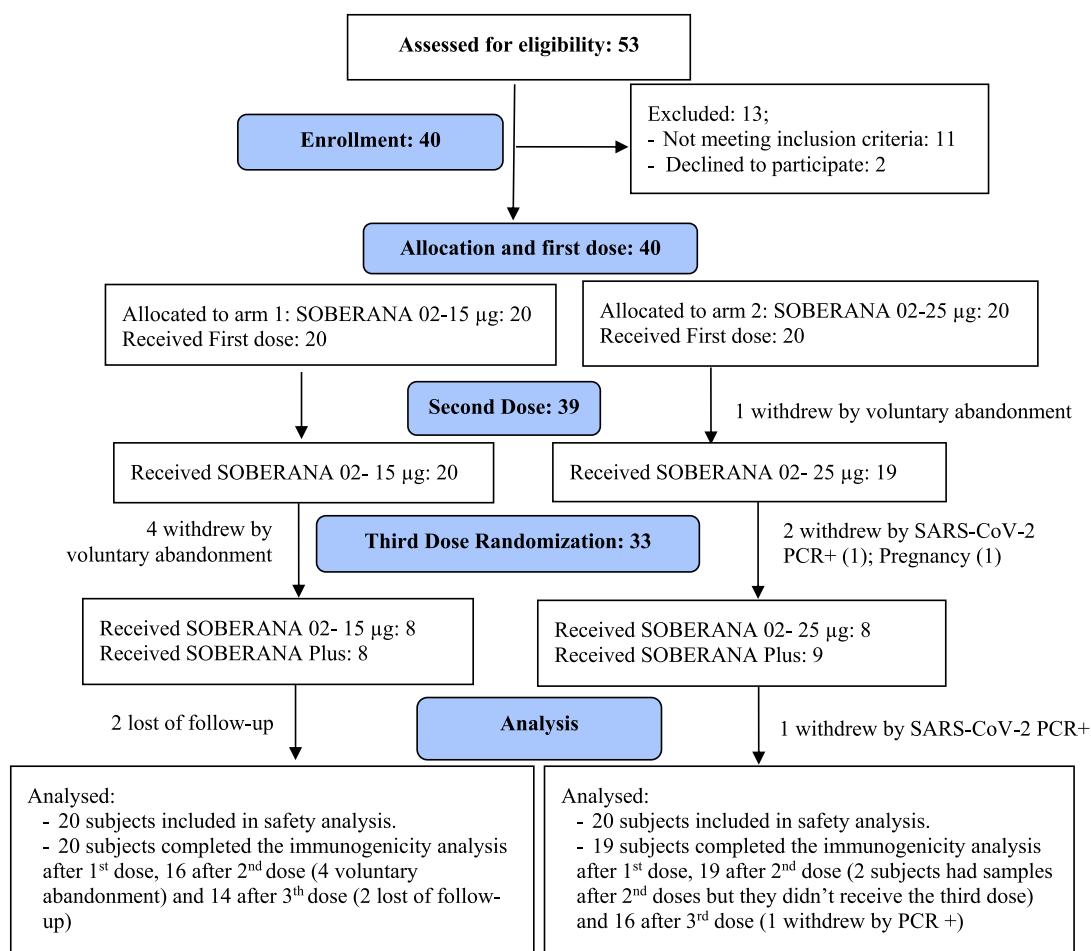


Fig. 1. Phase I Flow Chart. Footnote: Immunogenicity after 1st, 2nd or 3rd dose refers to 28 days after each dose.

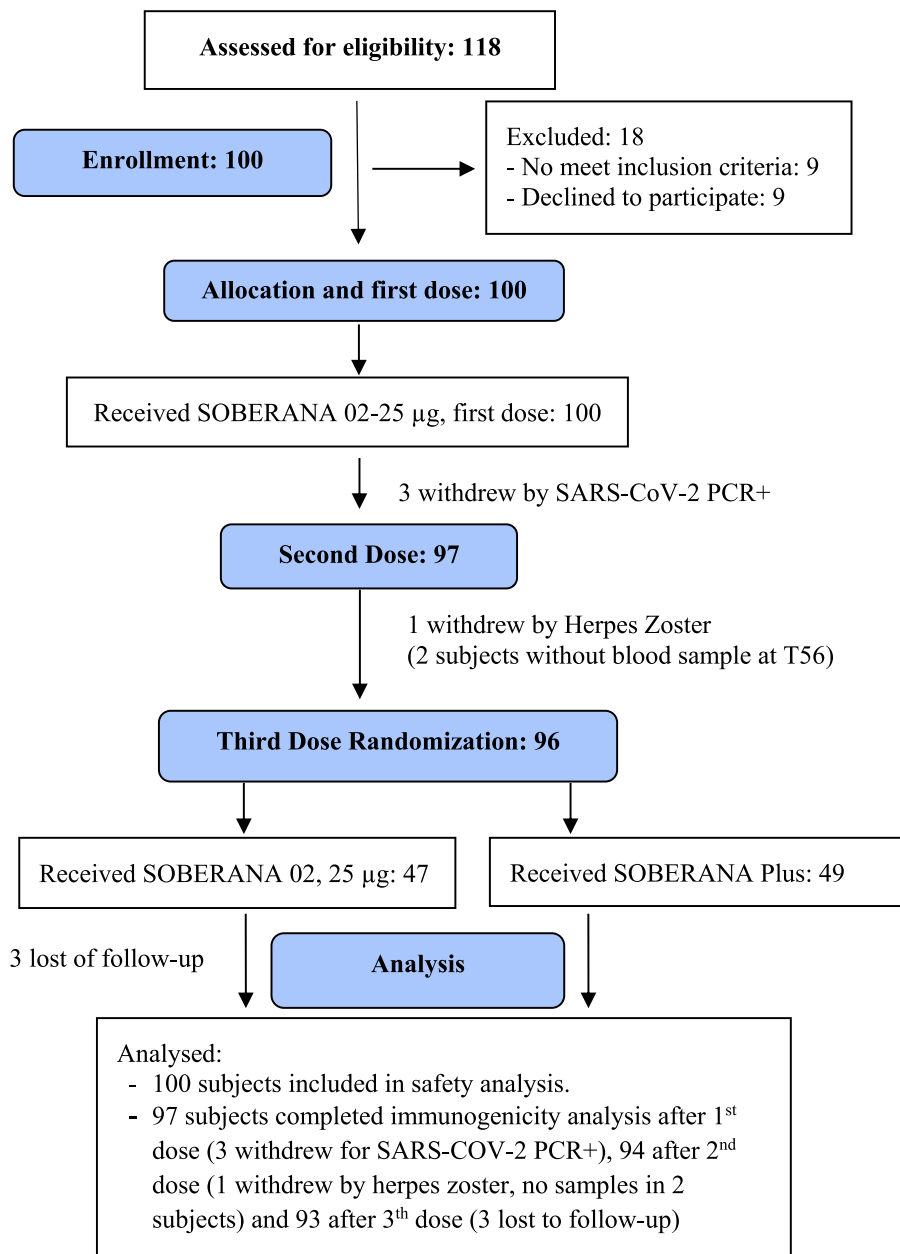


Fig. 2. Phase IIa Flow Chart. Footnote: Immunogenicity after 1st, 2nd or 3rd dose refers to 28 days after each dose.

phase IIa). Medical visits were planned after each dose at 24, 48 and 72 h, and on days 7 (in phase I), 14 and 28 (in phase I and IIa). Adverse event (AEs) were self-registered by the participants on a diary card and recorded during medical visits.

For evaluating immunogenicity, serum samples were collected on days 0 (before vaccination), 14, 28, 42, 56, 70 and 84 (corresponding 14 and 28 days after each dose). Peripheral blood mononuclear cells were collected for T-cell response evaluation, 28 days after the second and third dose in a participants subset.

2.5. Outcomes for safety evaluation

Both in phase I and phase IIa, the primary outcome was the occurrence of serious AEs measured daily during 28 days after each dose. Serious adverse event was defined as any untoward medical occurrence that is fatal, life-threatening, results in persistent or

significant disability/incapacity, requires hospitalization of the patient or prolongation of current hospitalization.

The secondary safety outcomes were solicited local and systemic AEs (measured daily during 7 days after each immunization) and unsolicited AE (measured daily during 28 days after each dose).

Solicited local AEs at the injection site included local pain, erythema, swelling, induration and local temperature; solicited systemic AEs were fever, general discomfort and rash. Other events were self-recorded throughout the 28 days follow-up period. Clinical laboratory test included pre-vaccination and post-vaccination biochemical serum analysis.

AEs were classified as serious or not. Also, AE severity was graded as mild (transient or mild discomfort, no interference with activity), moderate (mild to moderate limitation in activity), or severe (marked limitation in activity) according to Brighton Collaboration definition and the Common Terminology Criteria for

Adverse Events version 5.0 [25]. AEs were reviewed for causality and classified according to WHO: inconsistent causal association to immunization, consistent causal association to immunization, undetermined, unclassifiable [26].

2.6. Outcomes for immunogenicity evaluation

Other secondary outcomes were vaccine immunogenicity, seroconversion, kinetics of anti-RBD IgG production (on days 0, 14, 28, 42, 56, 70 and 84), neutralizing antibody titres (on days 0, 56 and 84) and inhibition of RBD-ACE2 interaction (on days 0, 14, 28, 42, 56, 70 and 84). Outcomes are detailed in [Supplemental Material](#), Appendix A-3). All immunological evaluations were performed by external laboratories.

Anti-RBD IgG response: Anti-RBD IgG in sera was evaluated by a quantitative ultramicro ELISA (UMELISA SARS-CoV-2 anti-RBD, Centre for Immunoassay, Havana, Cuba). The concentration of anti-RBD IgG was expressed as AU/mL. The seroconversion rate was calculated by dividing the concentration at each time point (at Tx) by the pre-vaccination concentration (at T0). A rate ≥ 4 was considered as seroconversion as reported for others vaccines [27,28]. ([Supplemental Material](#), Appendix C.1).

Molecular virus neutralization test: This ELISA is an *in-vitro* surrogate of the live-virus neutralization with some modifications [29]. An alternative molecular virus neutralization test using δ -variant L452R + T478K RBD displayed on phages was also evaluated for this variant of SARS-CoV-2 ([Supplemental Material](#), Appendix C.2, C.3 and C.4) [30,31].

Conventional virus neutralization test: Neutralizing antibodies against live D614G SARS-CoV-2 strain was performed by the conventional virus neutralization test, following the recommendation of Manenti & cols [32]. It is colorimetric assays based on the virus neutralization by antibodies, avoiding the cytopathic effect on VeroE6 cells. The neutralization titre represents the highest serum dilution giving 50% reduction of cytopathic effects. D614G strain was used for the test ([Supplemental Material](#), Appendix C.5).

Specific T-cell response: RBD-specific T-cell response producing IFN- γ and IL-4 were quantified with enzyme-linked immunospot (ELISpot) assay using human IFN- γ ELISpot^{PLUS} HRP kit (Mabtech, Sweden) and human IL-4 ELISpot^{PLUS} HRP kit (Mabtech, Sweden) following the manufacturer's instructions. Specific T-cell response was expressed as the number of spot-forming cells per 10^6 cells ([Supplemental Material](#), Appendix C.6).

Human Serum Convalescent Panel: A panel of convalescent serum samples (Cuban Convalescent Serum Panel, CCSP) was made with sera from 68 patients recovered from COVID-19 (diagnosed by positive PCR) on March–November 2020, during the first epidemic peak in Cuba (13 with severe disease, 30 with mild disease and 25 asymptomatic). All patients gave written consent to the Cuban National Centre of Medical Genetics in Havana, allowing the use of their samples for epidemiological research. This panel was characterized by anti-RBD IgG concentration (UA/ml), inhibition of RBD-hACE2 interaction (% of inhibition and molecular neutralization titre) and virus neutralization titre (cVNT50) with the same analytical methods used for vaccinated subjects in the clinical trials, except assay with phages [23].

2.7. Statistical analysis

Sample size calculation was done considering a serious vaccine related AE rate < 5% (for phase I) and < 1% (for phase IIa). Two-sided 95% confidence intervals for these proportion were calculated, taking into account a target width of 0.250 (for phase I) and 0.054 (for phase IIa). As consequence, the sample size by arm was 20 for phase I and 100 for phase IIa.

Safety and reactogenicity endpoints are described as frequencies (%). Demographic characteristics and AE data are reported as mean, standard deviation (SD), median, interquartile range, and range. Seroconversion rates for IgG antibodies anti-RBD (≥ 4 -fold increase in antibody concentration over baseline) were calculated. Anti-RBD IgG concentration, inhibition (%) of RBD-ACE2 interaction and cytokine-expressing cells were represented as median with interquartile range. Molecular neutralization titre (mVNT₅₀) and conventional virus neutralization titre (cVNT₅₀) are represented as geometric mean (GMT) and 95% confidence intervals (CI). Spearman's rank correlation was used to assess relationships among techniques used to evaluate the immune response. The Student's t-Test or the Wilcoxon Signed-Rank Test were used for before-after statistical comparison. Statistical analyses were done using SPSS version 25.0; R version 3.2.4; EPIDAT version 4.1 and Prism GraphPad version 6.0. An alpha signification level of 0.05 was used.

An Independent Data Monitoring Committee formed by external members (four members in phase I and six in phase II) specialized on clinical practice, epidemiology and statistics provided safety supervision and interim analyses. During phase I, three interim analyses were planned for safety: seven days after the first and the second dose of SOBERANA 02–15 μ g and seven days after first dose of SOBERANA 02–25 μ g. An interim analysis of immunogenicity was planned 14 days after the first dose in both groups. The final data analysis was done 28 days post third immunization.

3. Results

Phase I: from 53 individuals recruited for inclusion and screened from November 2th to 12th, 2020, 40 participants were selected ([Fig. 1](#)). Once the safety interim analysis for the group receiving SOBERANA 02–15 μ g showed no serious AEs, the second group received SOBERANA 02–25 μ g. Other two interim analyses (7 days after the second dose in the 15 μ g-group and 7 days after the first dose in the 25 μ g-group) showed no serious AEs. On day 56 half of individuals received a third dose of SOBERANA 02 (same dosage), half received a heterologous third dose of SOBERANA Plus-50 μ g.

Phase IIa: from 118 individuals recruited for inclusion and screened from December 17th 2020 to January 6th, 2021; 100 selected subjects received the first dose of SOBERANA 02–25 μ g and 97 of them received the second dose. For the third dose, 96 subjects (one withdrew by voluntary abandonment) were randomized for receiving SOBERANA 02–25 μ g (47 subjects) or SOBERANA Plus (49 subjects) ([Fig. 2](#)). Demographic characteristics are summarized in [Table 2](#). The mean age of participants was 38.2 years (SD 10.3) in phase I and 46.7 (SD 15.8) in phase IIa.

Adverse events: Thirty of 40 participants in phase I (75%) and 93 of 100 in phase IIa completed the three-dose scheme and follow-up visits. In phase I, 16 (40%) reported at least one AE within 28 days after vaccination. In the group receiving SOBERANA 02–25 μ g, 50% of subjects reported AEs compared to 30% in the group receiving SOBERANA 02–15 μ g; none reported serious or severe (grade 3) vaccine-related AEs. In phase IIa, 32 participants (32%) reported at least one AE within 28 days after vaccination; none reported serious vaccine-related AEs and only one reported two severe (grade 3) vaccine-related AEs (induration and erythema, [Tables 3 and 4](#)).

[Table 4](#) summarizes the frequency of subjects with solicited AEs. In phase I, local pain was reported in three subjects receiving SOBERANA 02–25 μ g (15%). Other events were systemic and unsolicited. The most frequent unsolicited AE in both treatment groups was high blood pressure (15% and 25% respectively) ([Supplemental Material](#), Appendix B, Table I); of all AEs 70% (arm 1: 15 μ g) and 84.6% (arm 2: 25 μ g) were classified as mild ([Supplemental Mate-](#)

Table 2
Demographic characteristics of participants in phase I and phase IIa clinical trials.

Demographic characteristics	Phase I			Phase IIa	
	Arm 1: SOBERANA 02–15 µg		Arm 2: SOBERANA 02–25 µg	Overall Phase I	SOBERANA 02–25 µg
	N (%)	N (%)	N (%)	N (%)	N (%)
Total (N)	20 (100)	20 (100.0)	40 (100.0)	100 (100.0)	
Sex	Female	5 (25.0)	10 (50.0)	15 (37.5)	57 (57.0)
	Male	15 (75.0)	10 (50.0)	25 (62.5)	43 (43.0)
Ethnicity	White	17 (85.0)	12 (60.0)	29 (72.5)	61 (61.0)
	Black	3 (15.0)	2 (10.0)	5 (12.5)	8 (8.0)
	Mixed race	0 (0.0)	6 (30.0)	6 (15.0)	31 (31.0)
Age	Mean (SD)	39.5 (10.4)	38.9 (10.5)	38.2 (10.3)	46.7 (15.8)
	Median (IQR)	39.0 (20.0)	38.5 (22.0)	39.0 (20.0)	48.5 (26.0)
	Range	(25; 58)	(24; 55)	(24; 58)	(19; 74)
Weight(kg)	Mean (SD)	74.2 (9.4)	68.3 (11.0)	71.3 (10.5)	73.4 (13.9)
	Median (IQR)	75.5 (12.0)	65.0 (10.0)	71.0 (15.3)	74.0 (20.0)
	Range	(50; 86)	(53; 101)	(50; 101)	(49; 115)
Height(cm)	Mean (SD)	168.2 (7.4)	165.4 (9.1)	166.8 (8.3)	166.0 (9.4)
	Median (IQR)	169 (11.0)	164 (15.0)	167 (13.0)	165.0 (13.0)
	Range	(150; 179)	(154; 186)	(150; 186)	(150; 198)
BMI kg/m ²	Mean (SD)	25.9 (1.9)	24.7 (2.9)	25.3 (2.5)	26.5 (3.4)
	Median (IQR)	26.0 (1.7)	24.5 (4.7)	25.8 (3.4)	26.9 (5.4)
	Range	(21.5; 29.4)	(20.0; 29.4)	(20.0; 29.4)	(19.1; 33.9)

SD = Standard Deviation. IQR = Interquartile range BMI = Body mass index.

Table 3
Phase I and phase IIa safety profile.

	Phase I		Phase IIa
	Arm 1: SOBERANA 02–15 µg N = 20 n(%)	Arm 2: SOBERANA 02–25 µg N = 100 n(%)	SOBERANA 02–25 µg N = 100 n(%)
Subjects with at least one AE	6 (30.0)	10 (50.0)	32 (32.0)
Subjects with at least one vaccine-related AE	2 (10.0)	7 (35.0)	28 (28.0)
Subjects with serious AE	0 (0)	1 (5.0)	2 (2.0)
Subjects with serious vaccine related AE	0 (0)	0 (0)	0 (0)
Subjects with severe (grade 3) AE	1 (5.0)	1 (5.0)	2 (2.0)
Subjects with severe (grade 3) vaccine related AE	0 (0)	0 (0)	1 (1.0)
Overall of reported adverse events			
Total of reported EA	10	13	72
Vaccine related AE	2 (20.0)	10 (76.9)	65 (90.3)
Serious AE	0 (0)	1 (7.7)	2 (2.8)
Serious vaccine related AE	0 (0)	0 (0)	0 (0)
Severe (grade 3) AE	1 (10.0)	1 (7.7)	3 (4.2)
Severe (grade 3) Vaccine related AE	0 (0)	0 (0)	2 (2.8)
Deaths	0 (0)	0 (0)	0 (0)

Note: Safety profile includes AEs after the third dose without distinction between homologous or heterologous dose. Percentage of vaccine related AE, serious AE, serious vaccine related AE, Severe AE and serious vaccine related AE correspond to the total of reported AE.

rial, Appendix B, Table II). In Phase IIa, pain at the injection site was also the most frequent solicited AE (in 22% of subjects). Other solicited and unsolicited AEs had frequencies < 5%. Headache was the most frequent vaccine-related, unsolicited AE (SM, Appendix B, Table II); of all AEs, 90.3% were classified as mild and 77.8% lasted < 24 h. No serious related adverse event or death were reported during phases I and IIa. (Supplemental Material, Appendix B, Table III). No clinically relevant changes were observed in haematology and blood chemistry analyses (Supplemental Material, Appendix B, Table IV). The number of participants reporting AEs decreased with the number of doses. AEs behaved similarly in both age subgroups (19–59 and 60–80 years) after SOBERANA 02 first, second and third (in homologous scheme) doses; after

Table 4
Solicited AEs during phase I and phase IIa.

	Phase I		Phase IIa
	Arm 1: SOBERANA 02–15 µg N = 20 n (%)	Arm 2: SOBERANA 02–25 µg N = 20 n (%)	SOBERANA 02–25 µg N = 100 n (%)
Subjects with solicited AEs			
Any	0	3 (15.0)	25 (25.0)
Severe (Grade 3)	0	0	1 (1.0)
Serious	0	0	0
Subjects with solicited systemic AEs			
Any	0	0	6 (6.0)
Severe (Grade 3)	0	0	0
Serious	0	0	0
General discomfort	0	0	5 (5.0)
Severe (Grade 3)	0	0	0
Serious	0	0	0
Rash	0	0	1 (1.0)
Severe (Grade 3)	0	0	0
Serious	0	0	0
Subjects with solicited local AEs			
Any	0	3 (15.0)	22 (22.0)
Severe (Grade 3)	0	0	1 (1.0)
Serious	0	0	0
Injection-site pain	0	3 (15.0)	22 (22.0)
Severe (Grade 3)	0	0	0
Serious	0	0	0
Erythema	0	0	4 (4.0)
Severe (Grade 3)	0	0	1 (1.0)
Serious	0	0	0
Local Warm	0	0	4 (4.0)
Severe (Grade 3)	0	0	0
Serious	0	0	0
Induration	0	0	3 (3.0)
Severe (Grade 3)	0	0	1 (1.0)
Serious	0	0	0
Swelling	0	0	3 (3.0)
Severe (Grade 3)	0	0	0
Serious	0	0	0

the heterologous third dose, the 60–80 years subgroup reported more solicited local and systemic AE than the 19–59 subgroup (Fig. 3, data from Phase IIa).

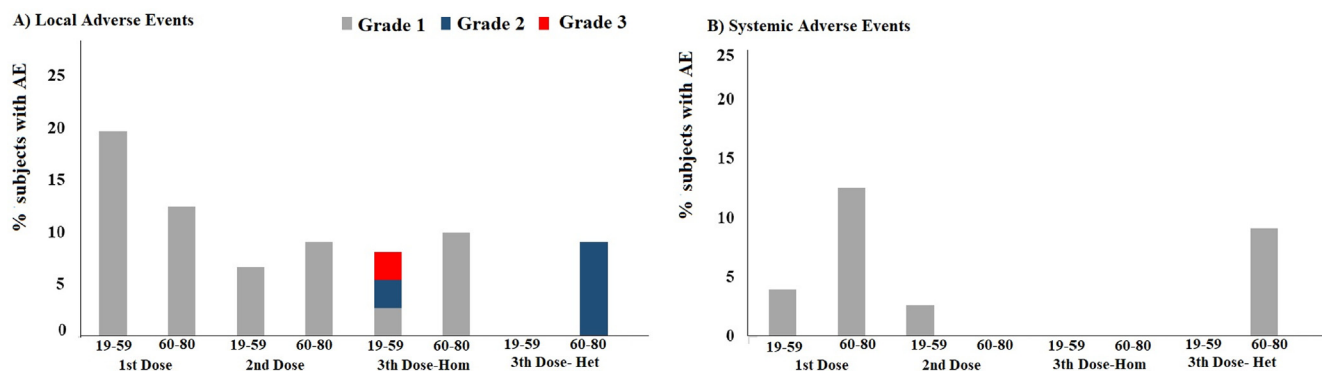


Fig. 3. Solicited local and systemic adverse event after each dose by age subgroups (Phase IIa).

Immunogenicity: In phase I, 28 days after second dose (day 56) both formulations of SOBERANA 02 induced seroconversion in $\geq 75\%$ participants. After the third dose (day 84) seroconversion increased to 85.7% with the homologous third dose and to 100% after the heterologous third dose (SOBERANA Plus) (Supplemental Material, Appendix B, Table V).

After two doses, the median of anti-RBD IgG concentration in subjects vaccinated with SOBERANA 02–15 μg was 25.9 (25th–75th percentile 14.9; 39.5); in those vaccinated with SOBERANA 02–25 μg the median was 40.3 (25th–75th percentile 18.5; 102.9) (Supplemental Material, Appendix B, Table V). Molecular inhibition of RBD:hACE2 interaction (expressed as % inhibition) and molecular virus neutralization (expressed as virus neutralization titre 50%) were higher in the 25 μg - than in the 15 μg -group. Virus neutralization titre was 5.8 (95% CI 4.5; 7.5) after two doses of 15 μg , it was 21.7 (95% CI 7.8; 60.3) after two doses of 25 μg (Supplemental Material, Appendix B, Table V).

In all participants, the third dose increased the IgG concentration ($p < 0.05$) as compared with the second dose. The combination of two doses of SOBERANA 02–25 μg with the heterologous third dose (SOBERANA Plus) also improved antibody functionality as compared with the homologous scheme: median of % inhibition of RBD:hACE2 interaction increased from 60.9% (25th–75th percentile 11.9; 87.6) to 89.2% (25th–75th percentile 57.2; 94.2), the GMT of molecular virus-neutralization titre (mVNT_{50}) increased from 94.5 (95% IC 18.5; 481.2) to 340 (95% IC 125.8; 918.5) and the conventional live-virus neutralization increased from 24.2 (95% IC 9; 65.3) to 65.6 (95% IC 22; 195.8) (Supplemental Material, Appendix B, Table V).

Given the interim safety and preliminary immunogenicity phase I results (data not shown), phase IIa participants received SOBERANA 02–25 μg in first and second immunizations, followed by homologous or heterologous third immunization. The study included participants up to 80 years in both schemes. The results were quite similar to those from phase I: 75% of participants seroconverted after the second dose and $\geq 95\%$ after the third, with significant increment ($p < 0.05$) in anti-RBD IgG titre, higher % inhibition of RBD:hACE2 interaction, molecular and virus neutralization titres. Better immunological results were obtained for the heterologous as compared to the homologous scheme (Supplemental Material, Appendix B, Table V).

Pooled data from all participants (in phases I and IIa) treated under the same vaccination scheme, two doses of SOBERANA 02–25 μg followed by either the homologous or the heterologous third dose show that the proportion of participants that seroconverted increased from 76.1% after two doses (day 56) to 98.3% or 98% respectively after the third heterologous or homologous dose (day 84) (Supplemental Material, Appendix B, Table VI). A significant increase ($p < 0.0005$) of anti-RBD antibodies was observed

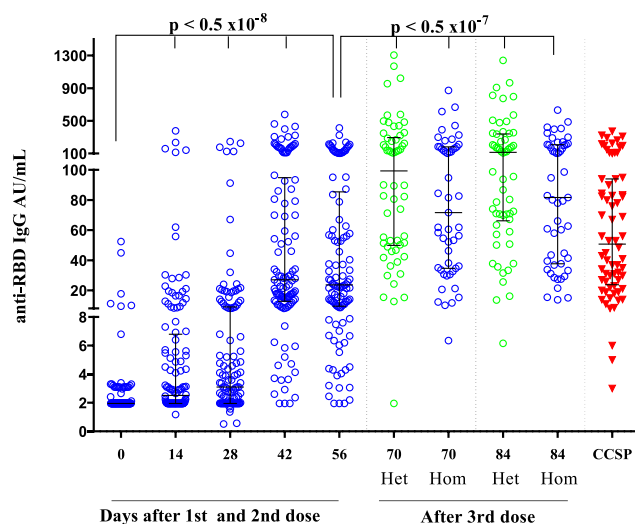


Fig. 4. Kinetics of anti-RBD IgG production after two doses of SOBERANA 02–25 μg and a third homologous or heterologous dose (pooled analysis from phase I and phase IIa). FOOTNOTE: Subjects 19–80 years-old received two doses (T0, T28) of SOBERANA 02–25 μg and a third dose (T56) homologous (Hom: blue points) or heterologous with SOBERANA Plus (Het: green points). Anti-RBD IgG concentration is expressed in arbitrary units/mL (median, 25th–75th percentile). CCSP: Cuban Convalescent Serum Panel (red triangles). p values represent the statistic differences with T0 or T56 as indicated, using Wilcoxon signed rank test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

after first (day 14) and second doses (day 42) as compared with pre-vaccination (Fig. 4). For both third dose subgroups, on day 84 the IgG level was significantly superior ($p < 0.0005$) to its value on day 56; the highest increase was observed in subjects with the heterologous third dose (on day 84, the median with heterologous scheme was 4.7-fold higher than on day 56; whilst with the homologous scheme the increase was 3.4-fold). Also, after the heterologous third dose, the median IgG value was 2.2-fold higher than the median for the Cuban Convalescent Serum Panel (CCSP) (1.6-fold higher after the homologous third dose) (Fig. 4; Supplemental Material, Appendix B, Table VI).

Elicited anti-RBD antibodies inhibited the interaction of RBD with the human ACE2 receptor. There was a significant increase in % inhibition ($p < 0.0005$) after the second dose (day 42) compared to pre-vaccination and after both alternative third doses compared to day 56 (Fig. 5A). After the third dose (day 84, considering together homologous and heterologous third dose), the inhibition median was 78.9% (25th–75th percentile 53.6; 91.1) and GMT of molecular neutralization titre was 257.7 (95% IC 203.2;

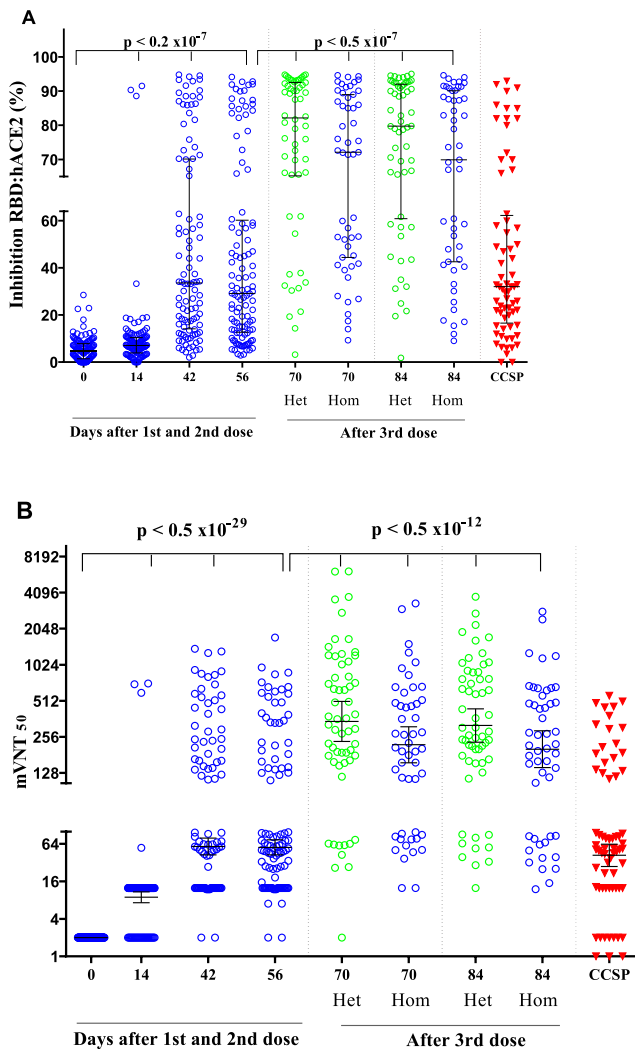


Fig. 5. Anti-RBD IgG antibodies inhibit the interaction between RBD and human ACE2 receptor after two doses of SOBERANA 02-25 µg and a third homologous or heterologous dose (pooled analysis from phase I and phase IIa). FOOTNOTE: Subjects 19–80 years old received two doses (T0, T28) of SOBERANA 02-25 µg and a third dose (T56) homologous, (Hom: blue points) or heterologous with SOBERANA Plus (Het: green points). A: % inhibition of RBD:hACE2 interaction at 1/100 serum dilution (median, 25th-75th percentile). B: Molecular virus neutralization titre mVNT₅₀: highest serum dilution inhibiting 50% of RBD:hACE2 interaction; (GMT, IC 95%). CCSP: Cuban Convalescent Serum Panel (red triangles). p values represent statistic differences with T0 or T56, as indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

326.9), both significantly higher ($p < 0,0005$) than those attained after the second dose (data not shown). However, the heterologous third dose showed an mVNT₅₀ increase of 5.7-fold, the homologous scheme increased 3.6-fold respect to the second dose (Fig. 5A, 5B; Supplemental Material, Appendix B, Table VII). As observed in Fig. 5B, GMT of mVNT₅₀ after two doses was similar to the value for CCSP, and it was higher after the third dose, particularly after the heterologous immunization.

The conventional virus neutralization titre (cVNT₅₀) was evaluated pre-vaccination and 28 days after the second and third doses (Fig. 6). After two doses, the GMT reached 12.5 (95% IC 9.6; 16.1), significantly increasing ($p < 0,0005$) to 37.5 (95% IC 29.8; 47.3) after the third dose. There were no significant differences for GMT cVNT₅₀ (heterologous: 42.5, 95% IC 30.4; 59.4), homologous: 32.8, 95% IC 23.8; 45.3); the heterologous third dose showed a cVNT₅₀ increase of 3.4-fold, the homologous scheme increased

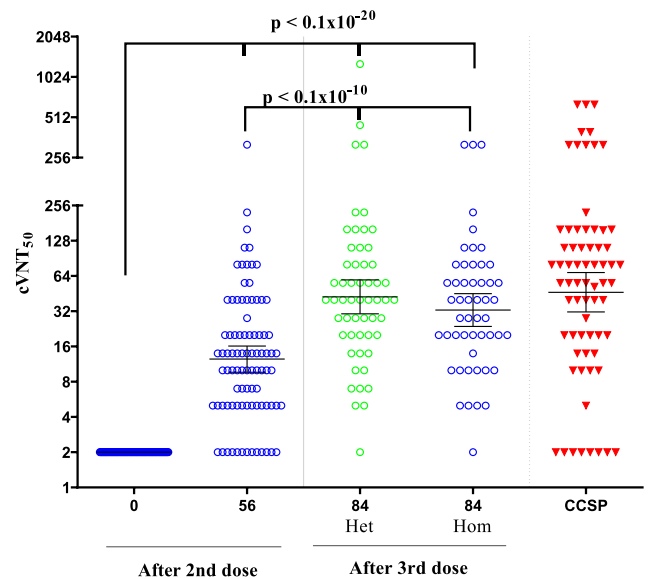


Fig. 6. Neutralizing titre of SARS-CoV-2 (D614G) live-virus after two doses of SOBERANA 02-25 µg and a third homologous or heterologous dose (pooled analysis from phase I and phase IIa). FOOTNOTE: Subjects 19–80 years old received two doses (T0, T28) of SOBERANA 02-25 µg and a third dose (T56) homologous, (Hom: blue points) or heterologous (SOBERANA Plus, Het: green points). cVNT₅₀: Conventional live-virus neutralization titre (GMT, IC 95%). CCSP: Cuban Convalescent Serum Panel (red triangles). p values represent the statistic differences with T0 or T56, as indicated, using paired Student *t* test with log-transformed variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

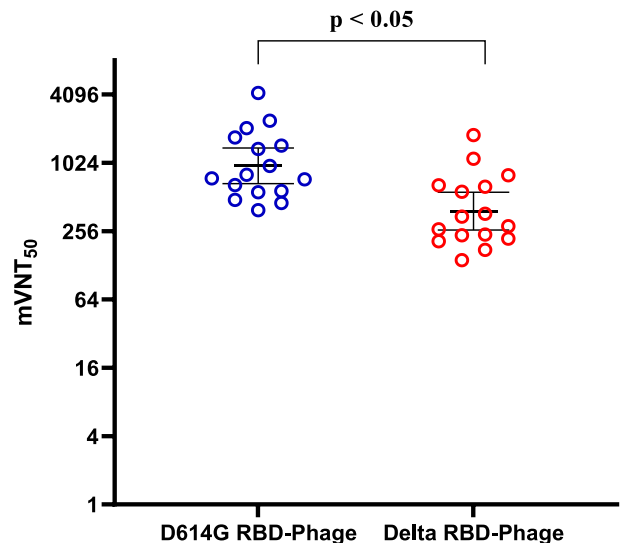


Fig. 7. Anti-RBD IgG antibodies inhibit the interaction between the human ACE2 receptor and phages displaying D614G-RBD or δ-RBD variant. FOOTNOTE: Sera from 16 individuals vaccinated with heterologous schedule were tested (GMT, IC 95%). During the trial clinical trials, the predominant circulating strain was D614G. p value represents the statistic differences as indicated, using paired Student *t* test with log-transformed variables.

2.6-fold. They were similar to the CCSP value (Fig. 6) (SM, Appendix B, Table VIII). The molecular neutralizing effect of anti-RBD IgG against phages displaying delta (δ)-RBD (L452R + T478K) compared to D614G-RBD was analysed in 16 serum samples from individuals vaccinated with the heterologous scheme. Fig. 7 shows an mVNT₅₀ GMT of 962.9 (95% IC 670.1; 1384) against phages displaying D614G-RBD and 384.1 (95% IC 262; 562.9) against δ-RBD phages,

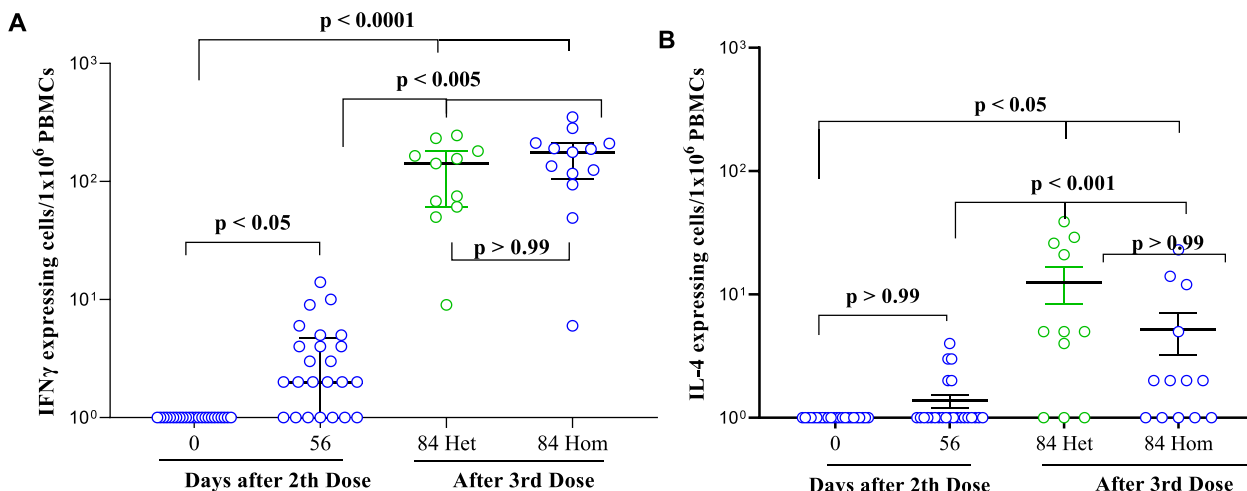


Fig. 8. IFN- γ - and IL-4-secreting cells in peripheral blood mononuclear cells stimulated with RBD. FOOTNOTE: Subjects 19–80 years old received two doses (on days 0, 28; N = 24) of SOBERANA 02-25 μg and a third dose (on day 56) homologous (SOBERANA 02, Hom; blue points, N = 13) or heterologous (SOBERANA Plus, Het; green points; N = 11). p value represents the statistical differences as indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

meaning a reduction of 2.5-fold the molecular neutralization capacity of the antibodies.

Both age subgroups (19–59 and 60–80) in phase IIa showed similar immunological responses ($p \geq 0.05$) after the third dose, the neutralizing antibodies titres were similar. Significant differences only were observed for the molecular neutralization titre (mVNT₅₀) with higher values in the 19–59 years-group respect to 60–80 years-groups. (Supplemental Material, Appendix B, Table IX).

RBD-specific T cell response was assessed by IFN- γ and IL-4 expression in peripheral blood mononuclear cell (PBMC) in a subset of participants, as an indicative of Th1 or Th2 profile. After two doses of SOBERANA 02 (T56), the number of IFN- γ forming cells were statistically different ($p < 0.05$) to baseline levels (T0) (Fig. 8A). The number of IL-4 secreting cells did not increase ($p > 0.99$) (Fig. 8B) showing a classical profile of Th1 cellular immune response after two doses of SOBERANA 02. A significant increase of both IFN- γ producing cells ($p < 0.005$) and IL-4 producing cells although significant ($p < 0.001$) occurred after the third dose (day 84). There were no differences between both alternative third doses ($p > 0.99$).

There was a good correlation among all variables (coefficients greater than 0.7, Supplemental Material, Appendix B, Table X). The likelihood ratio (using Bayes Factor) was used as Benefit-Risk index. In all considered scenarios, there is strong evidence for benefit, with a higher index for the heterologous scheme (Supplemental Material, Appendix B, Figure 1).

4. Discussion

Conjugate vaccines have been used for more than 30 years, mainly in children, for preventing bacterial infection diseases. Their induction of potent B and T immune responses, both endowed with immunological memory, marked a breakthrough in vaccinology [33]. SOBERANA 02 is an innovative conjugate vaccine in which the viral antigen RBD is conjugated to tetanus toxoid. As far as we know, it is the first protein–protein conjugated vaccine to be used in humans.

In both the three-dose homologous and heterologous scheme SOBERANA 02 showed an excellent safety profile, with predominance of local over systemic AEs. The frequency of adverse events

(50% in phase I and 31% in phase IIa), particularly the systemic AEs, is lower compared to anti-SARS-CoV-2 mRNA or adenovirus-vectored vaccines [34–38]. These results provide the first evidences of safety of SOBERANA 02–25 μg in three doses or in heterologous combination with SOBERANA Plus. In phase I, 25 μg -dose SOBERANA 02 was more immunogenic than 15 μg -dose; in consequence, after phase I interim analysis the high dose progressed to phase II trial.

Vaccine candidates eliciting similar or higher immune response as compared with convalescents serum panels have moved forward in clinical evaluation [38,39,27]. The pooled immune response data from phase I and phase IIa were compared with those from the Cuban Convalescent Serum Panel (CCSP). Two doses of SOBERANA 02–25 μg elicited similar immune response compared to the CCSP in terms of anti-RBD IgG titre and molecular inhibition of RBD:hACE2 interaction; however, elicited RBD antibodies showed a lower viral neutralization capacity. For this reason, the study incorporated a third dose. Both the homologous and the heterologous—incorporating SOBERANA Plus— three-dose schemes, increased the humoral immune response and titre of neutralizing antibodies. In both cases, neutralizing capacities were similar to the observed in convalescents.

The humoral immune response by age group was explored in phase IIa and it included a small number of subjects aged 60–80. The results presented herein are encouraging as this age group is severely affected by COVID-19 [40].

By mid-2021, the SARS-CoV-2 VOC δ became predominant worldwide, being 60% more transmissible than variant α [41] and reducing vaccine efficacy towards the onset of symptomatic disease [42,43]. The predominant variant circulating in Havana at the moment of these studies was D614G, but it was replaced firstly by beta (March–June 2021) and later completely by delta (July–October 2021) [44]. We evaluated molecular neutralizing capacity (mVNT₅₀) against VOC δ and found a decrease of 2.5-fold compared to molecular neutralizing capacity against D614G variant. This result is in correspondence with reports of three- to five-fold reduction in neutralization titres against VOC δ in respect to VOC α in sera from individuals immunized with mRNA vaccines or adenoviral-vectors [45]. Protection against the circulating VOCs will be addressed in the next clinical trials.

Specific T cell response plays an important role for anti-SARS-CoV-2 immunity [46,47]. Our results demonstrate the activation

of Th1 cellular immune pattern after two doses of SOBERANA 02, characterized by predominant IFN- γ - over IL-4-secreting cells. After third dose in homologous or heterologous scheme, both cytokines-secreting cells increased significantly, predominating IFN- γ secretion, suggesting a balanced Th1/Th2 profile that contribute to the high increase in anti-RBD IgG levels.

The first heterologous scheme in COVID vaccine was reported for Sputnik V (two shots scheme with different adenoviral vector) [7]. Recent studies are evaluating heterologous booster effects of mRNA BNT162b2 in individuals previously vaccinated with adenoviral vaccines and other vaccine combinations as prime/boost heterologous strategy [48]. Our approach of heterologous 2 + 1 scheme was different. We focused in priming with two doses (0, 28 days) with RBD-tetanus toxoid conjugate vaccine SOBERANA 02 for inducing specific humoral and cellular immune response favoured by the multiepitopic presentation of RBD [1920]; followed by a third dose (on day 56) with SOBERANA Plus (dimeric-RBD/alumina), changing the RBD epitope presentation to the immune system. Although the sample size did not allow for statistical comparisons between heterologous and homologous schemes, these results encouraged us to move to phase IIb and phase III trial with the heterologous scheme. Both three-dose schemes were equally safe; in contrast to a recent report where heterologous boost of mRNA vaccine in individuals previously vaccinated with ChAdOx1 (ChAd) increased systemic reactivity as compared to homologous boost with ChAdOx1 (ChAd) [49].

The main limitation of our study is its open label design; the lack of a control-placebo group precludes the comparison with unvaccinated subjects.

In conclusion, SOBERANA 02 is safe, well tolerated and immunogenic in adult aged 19–80 years. Application of a heterologous scheme with SOBERANA Plus increased the immune response with excellent safety profile. These results pave the way for further evaluation of the heterologous scheme in phase IIb and phase III clinical trials.

Contributor Roles

METR was the principal investigator and LVS was the co-principal investigator of this trial. METR, MCR, BPM, CVS, YVB, DGR and VVB conceived the study, designed the trial, the study protocol, and were involved in data analysis and interpretation. SFC, YCR, DSM, URG, TBA, EOM, DGR and GWC contribute to the vaccine design and batches production. LRN, BSR, RPN, THG, GBB, FPE, OCS, AFQ, MGM, APD, GBR, BPM carried out immunological experiments and the analysis of results. CVS and RGM were involved in data curation and statistical analysis of data. DGR, CVS, YGV, SFC and VVB wrote the manuscript and the rest of authors provided paper feedback.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [The Finlay Vaccine Institute, the Centre of Molecular Immunology and the University of Havana have filed patent applications related to the vaccine SOBERANA 02. The authors declare the following competing financial interest(s): L.R.N, B.S.R, R.P.N, S. F.C, Y.C.R, D.S.M, U.R.G, T.B.A, E.O.M, D.G.R, Y.V.B., D.G.R, V.V.B are

co-inventors on provisional SARS-CoV-2 vaccine patents (Cu 2020-69). The rest of the authors declare no competing interests. No authors received an honorarium for this paper.]

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2022.05.082>.

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