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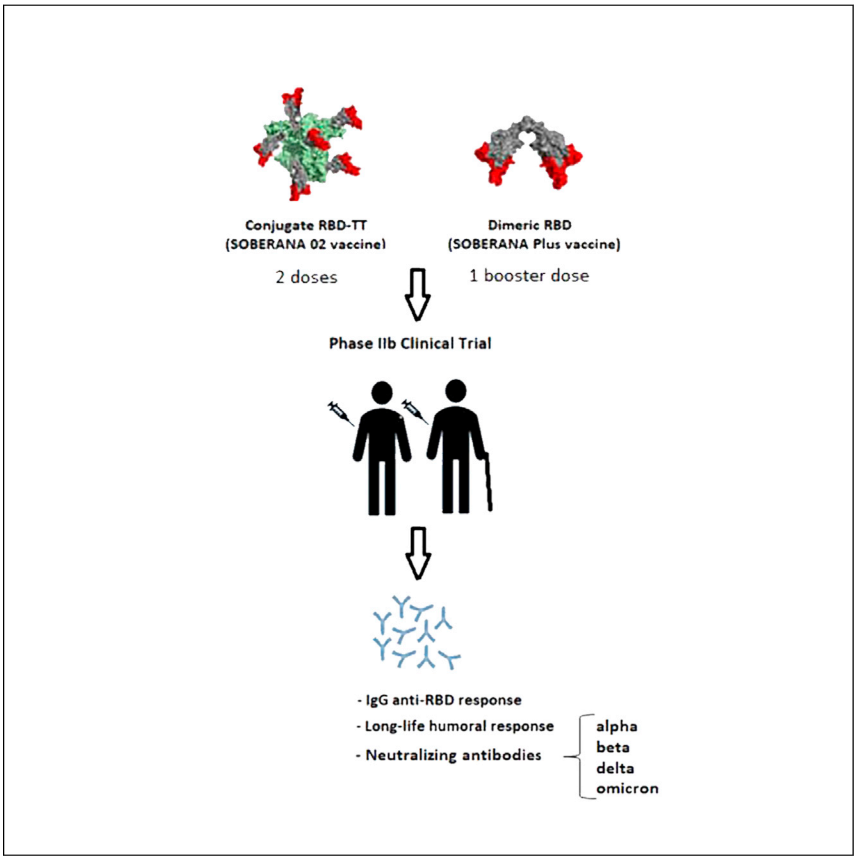
Safety and immunogenicity of anti-SARS-CoV-2 heterologous scheme with SOBERANA 02 and SOBERANA Plus vaccines: Phase IIb clinical trial in adults



Phase IIb of SOBERANA 02 vaccine candidate demonstrated its safety and immunogenicity in a two- or three-dose heterologous schedule with SOBERANA Plus in adults aged 19–80. Neutralizing antibodies against D614G were detected after 7–8 months. Neutralizing IgG antibodies were detected against D614G and VOCs Alpha, Beta, Delta, and Omicron.

Translation to Patients

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Highlights

Heterologous schedule was well tolerated in a cohort of 810 adults aged 19–80 years

Specific IgG and neutralizing antibody responses were observed after vaccination

Neutralizing GMT versus D614G variant persisted after 7–8 months of vaccination

Serum samples of vaccinees neutralized VOCs Alpha, Beta, Delta, and Omicron

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Safety and immunogenicity of anti-SARS-CoV-2 heterologous scheme with SOBERANA 02 and SOBERANA Plus vaccines: Phase IIb clinical trial in adults

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SUMMARY

Background: SOBERANA 02 has been evaluated in phase I and IIa studies comparing homologous versus heterologous schedule (this one, including SOBERANA Plus). Here, we report results of immunogenicity, safety, and reactogenicity of SOBERANA 02 in a two- or three-dose heterologous scheme in adults.

Method: Phase IIb was a parallel, multicenter, adaptive, double-blind, randomized, and placebo-controlled trial. Subjects (n = 810) aged 19–80 years were randomized to receive two doses of SARS-CoV-2 RBD conjugated to tetanus toxoid (SOBERANA 02) and a third dose of dimeric RBD (SOBERANA Plus) 28 days apart; two production batches of active ingredients of SOBERANA 02 were evaluated. Primary outcome was the percentage of seroconverted subjects with ≥4-fold the anti-RBD immunoglobulin G (IgG) concentration. Secondary outcomes were safety, reactogenicity, and neutralizing antibodies.

Findings: Seroconversion rate in vaccinees was 76.3% after two doses and 96.8% after the third dose of SOBERANA Plus (7.3% in the placebo group). Neutralizing IgG antibodies were detected against D614G and variants of concern (VOCs) Alpha, Beta, Delta, and Omicron. Specific, functional antibodies were detected 7–8 months after the third dose. The frequency of serious adverse events (AEs) associated with vaccination was very low (0.1%). Local pain was the most frequent AE.

Conclusions: Two doses of SOBERANA 02 were safe and immunogenic in adults. The heterologous combination with SOBERANA Plus increased neutralizing antibodies, detectable 7–8 months after the third dose.

Trial registry: https://rpcec.sld.cu/trials/RPCEC00000347

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CONTEXT AND SIGNIFICANCE

Conjugate vaccine candidate SOBERANA 02 (recombinant receptor-binding domain [RBD] conjugated to tetanus toxoid) was previously evaluated in open-label phase I and IIa clinical trials. Here, Toledo-Romani et al. report the results of a double-blind, placebo-controlled phase IIb trial assessing immunogenicity and safety of SOBERANA 02 in a three-dose heterologous schedule with SOBERANA Plus (recombinant RBD dimer) in adults aged 19–80. The authors found an excellent safety profile and high immunogenicity. Antibody functional response was still detected after 7–8 months of vaccination. Neutralizing antibodies were detected against Alpha, Beta, Delta, and Omicron variants of SARS-CoV-2 virus. The results obtained endorsed phase III clinical evaluation.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has led to an unprecedented effort in vaccine development, and several vaccines based on different platforms have received emergency-use authorization.[1] Despite the outstanding progress, equal access to vaccines continues being a major challenge.[2]

SOBERANA 02 is an anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine candidate which has the recombinant receptor-binding domain (RBD) protein as the immunogen conjugated to tetanus toxoid.[3,4] The phase I study evaluated safety, reactogenicity, and immunogenicity of SOBERANA 02 in 40 adults 19–59 years old and compared SOBERANA 02 in a three-dose schedule versus a heterologous schedule (two doses of SOBERANA 02 and a third dose of SOBERANA Plus; active pharmaceutical ingredient: RBD dimer).[5] After an interim analysis, the higher dose (SOBERANA 02, 25 μg) was selected for a phase II study[6] designed in two stages (IIa and IIb); IIa was an open trial evaluating the homologous and heterologous schedules in 100 adults (19–80 years).[7] A pooled analysis of phases I and IIa concluded that the heterologous scheme was safe, well tolerated, and elicited the highest immune response, with a mixed Th1/Th2 profile.[6] Here, we report immunogenicity, safety, and reactogenicity of two doses of SOBERANA 02 and the heterologous scheme with a third dose of SOBERANA Plus in a randomized, double-blind, placebo-controlled phase IIb clinical trial in adults 19–80 years old.

RESULTS

Flow chart and demographics

From mid-January to the end of February 2021, 948 individuals were recruited for the phase IIb trial; 138 were excluded and 810 included (Figure 1). Eligible participants were randomly assigned to receive the vaccine (two doses of SOBERANA 02 and one dose of SOBERANA Plus) or placebo at 28 day intervals. The demographic characteristics are summarized in Table 1. There were eight subjects, seven in vaccine and one in placebo group, with (BMI <18.5; BMI ≥34.9).

Immune response assessment

During recruitment, potential participants were screened using a qualitative rapid test for anti-SARS-CoV-2 antibodies; those with positive results were excluded. Pre-vaccination serum samples (at T0) were evaluated through quantitative anti-RBD immunoglobulin G (IgG) determination; 98.3% were seronegative for anti-RBD IgG (<8 AU/mL) (Figure 2A).

On day 14 after the first dose, the proportion of subjects with ≥4-fold increase in anti-RBD IgG concentration was significantly different ($p < 0.005$) in vaccine (20%) and placebo (3.8%) groups. These values increased to 76.3% (median 26.5 AU/mL) after the second dose (sample on T56) and 96.8% (median 122.2 AU/mL) after the third dose (samples collected either on T70 or T84), while values for placebo were 7.3% on T56. This represents a 4.8-fold increase in anti-RBD IgG concentration ($p < 0.0005$) after the third dose compared with the second (paired samples) and a 2.4-fold increase compared with the Cuban convalescent serum panel (CCSP). (Figure 2A; Table S1).

The molecular inhibition of RBD:hACE2 interaction (expressed as a percentage of inhibition and molecular virus neutralization titer [$mVNT_{50}$]) also increased. The inhibition median after two doses of SOBERANA 02 was 28.4% (25^{th}–75^{th} percentile 10.8; 67.0), similar to the value for CCSP (32%; 25^{th}–75^{th} percentile 26.6; 62.2). After the third dose, this value increased to 85.5% (25^{th}–75^{th} percentile 49.4; 93.1); the geometric mean titer (GMT) for $mVNT_{50}$ was 340.0 (95% confidence interval [CI]: 304.9; 379.0), which

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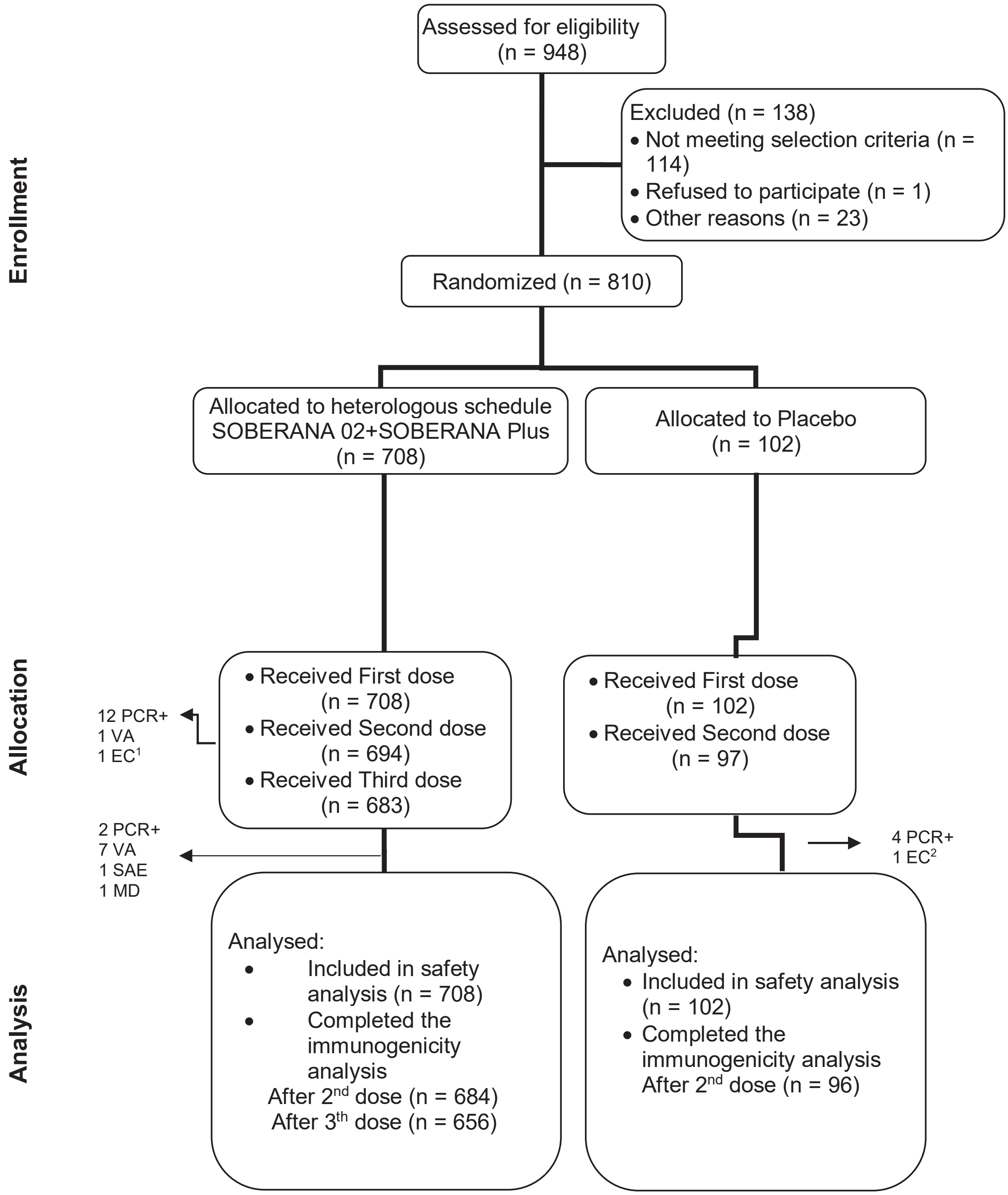

Figure 1. Flow chart of clinical trial phase IIb

EC, exclusion criteria ([1], history of unresolved neoplasm; [2], blood transfusion for megaloblastic anemia); MD, medical decision; VA, voluntary; SAE (multiform erythema).

represented a 5.4-fold increase compared with the value after the second dose (paired samples, $p < 0.0005$, Figures 2B and 2C) and a 6.9-fold increase, considering all samples in T70/T84, in mVNT$_{50}$ (289.0; 95% CI: 258.4; 323.4) compared with the CCSP value (Table S1).

cVNT$_{50}$ was evaluated in a subset of samples randomly selected from participants with seroconversion after the second (on T56) and third doses (on days T70 or T84). After two

Table 1. Baseline demographic characteristics of subjects included in the clinical trial

	Groups randomized according to vaccine candidate or placebo		
	Vaccine	Placebo	Total
N	708	102	810
Sex (%)			
Female	359 (50.7)	50 (49)	409 (50.5)
Male	349 (49.3)	52 (51)	401 (49.5)
Ethnicity (%)			
White	513 (72.5)	80 (78.4)	593 (73.2)
Black	84 (11.9)	9 (8.8)	93 (11.5)
Mixed race	111 (15.7)	13 (12.7)	124 (15.3)
Age (years)			
Mean (SD)	47.0 ± 15.8	47.1 ± 16.0	47.0 ± 15.8
Median (IQR)	48.0 ± 26.0	48.0 ± 25.0	48.0 ± 26.0
Range	19–80	19–80	19–80
19–59 (%)	544 (76.8)	78 (76.5)	622 (76.8)
60–80 (%)	164 (23.2)	24 (23.5)	188 (23.2)
Weight (kg)			
Mean (SD)	74.2 ± 14.9	73.9 ± 12.6	74.2 ± 14.6
Median (IQR)	72.0 ± 21.5	75.0 ± 18.0	73.0 ± 21.0
Range	42–120	40–110	40–120
Height (cm)			
Mean (SD)	167.4 ± 10.0	167.0 ± 9.2	167.3 ± 9.9
Median (IQR)	167.0 ± 14.0	165.5 ± 14.0	167.0 ± 14.0
Range	136–200	147–186	136–200
BMI (kg/m^2)			
Mean (SD)	26.4 ± 4.0	26.5 ± 3.8	26.4 ± 4.0
Median (IQR)	26.1 ± 5.7	26.2 ± 5.4	26.1 ± 5.7
Range	18.1–41.0[a]	17.3–34.7[a]	17.3–41.0[a]

Vaccine = heterologous scheme (SOBERANA 02 two doses + SOBERANA Plus). Vaccine data correspond to results from participants vaccinated with both API batches. Results for individual batches are presented below. Data are n (%) unless otherwise specified. Mean (SD), mean ± standard deviation; median (IQR), median ± interquartile range; BMI, body mass index.

[a]There were eight subjects, seven in vaccine and one in placebo group, with (BMI <18.5; BMI ≥34.9).

doses, the $cVNT_{50}$ GMT was 65.9 (95% CI: 46.9; 92.7), comparable to the CCSP value (GMT 41.8; 95% CI: 27.7; 63.2). After the third heterologous dose, a remarkable, statistically significant increase ($p < 0.0005$) was observed in 38 paired samples (GMT 219.2; 95% CI: 178.2; 269.7); this is a 3.6-fold increase in the value after the second dose (GMT 61.1; 95% CI: 41.4; 90.1, Figure 2D) and a 2.6-fold increase in the value of CCSP considering all tested samples in T70/T84 (Table S1).

Results of immunological determinations in the eight individuals with (BMI <18.5; BMI ≥34.9) are shown in Table S2. Of the seven subjects in the vaccine group, four (57.1%) seroconverted at T56 and six (85.7%) at T70/84. Immunologic results of these seven subjects were in the CI considering all subjects in vaccine group.

Neutralization of SARS-CoV-2 variants was analyzed in sera from 16 subjects that completed the vaccination schedule. $cVNT_{50}$ GMT was 370.4 (95% CI: 306.6; 447.5) against the D614G variant, whereas $cVNT_{50}$ GMTs against Alpha, Delta, Omicron, and Beta variants were 333.2 (95% CI: 269.7; 411.6), 156.3 (95% CI: 117.9; 207.2), 145.9 (95% CI: 100; 213.0), and 50.0 (95% CI: 29.4; 84.8), respectively (Figure 3). Compared with D614G, no differences were detected in neutralizing titers against the

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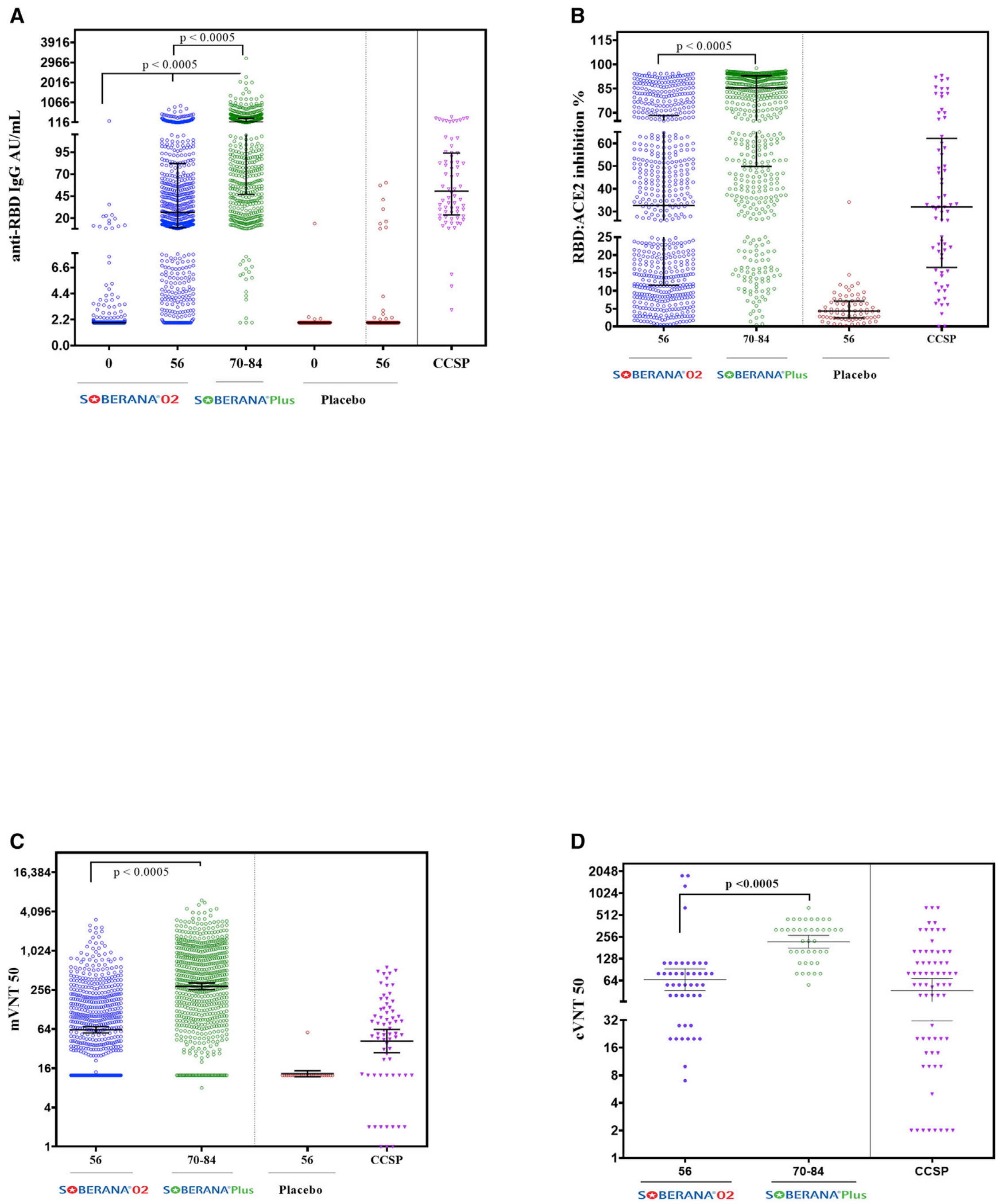

Figure 2. Immunogenicity after vaccination in subjects aged 19–80 years

(A) Kinetics of anti-RBD IgG concentration expressed in arbitrary units/mL (median, 25th–75th percentile).

(B) Percentage of inhibition of RBD:hACE2 interaction at 1/100 serum dilution (median, 25th–75th percentile).

(C) Molecular virus neutralization titer $mVNT_{50}$, highest serum dilution inhibiting 50% of RBD:hACE2 interaction (GMT, 95% CI).

(D) Conventional live-virus neutralization titer $cVNT_{50}$ (GMT, CI 95%) against SARS-CoV-2 D614G variant. Blue dots: response after two doses (on T0, T28) of SOBERANA 02. Green dots: response at T70 or T84 after receiving the third dose on T56, this one of SOBERANA Plus. Brown dots: subjects receiving placebo. CCSP (purple), Cuban convalescent serum panel. p value: statistic differences (on T70 or T84) compared with T56 or T0.

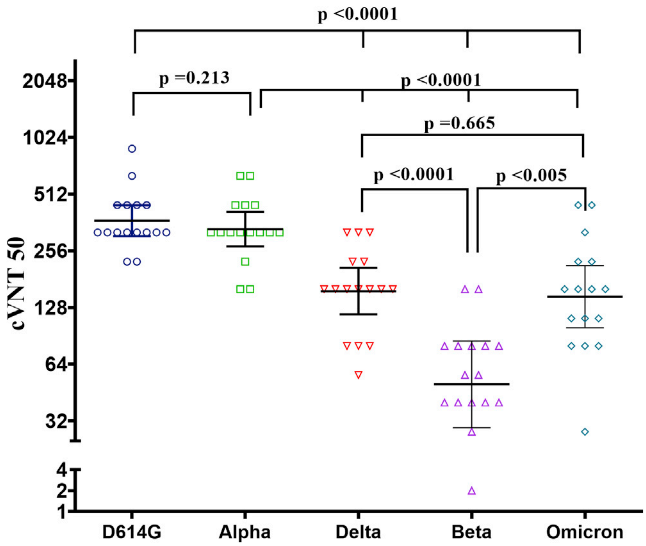

Figure 3. Live-virus neutralization titer against SARS-CoV-2 variants

Sera from 18 subjects vaccinated with complete schedule (two doses SOBERANA 02 + one dose SOBERANA Plus, 28 days apart) were evaluated ($cVNT_{50}$: GMT, 95% CI) against variants B.1.1.7 Alpha, B.1.617.2 Delta, BA1.21K Omicron, and B.1.351 Beta and compared with D614G variant. p values represent the statistic differences as indicated, using paired Student t test with log-transformed variables.

Alpha variant; however, there was a reduction of 2.4-, 2.5-, and 7.4-fold against Delta, Omicron, and Beta variants, respectively.

In females, in participants 19–59 years and in individuals without comorbidities, the analysis of immunological variables by participants' subgroups indicated a significant increase ($p < 0.00005$) in all variables except for $cVNT_{50}$ between sex subgroups (Table S3). Compared with placebo, in the vaccine group, there was a significant increase in the immune response for all subgroups (data not shown).

There was a good correlation among all variables (coefficients >0.8) except for $cVNT_{50}$ after the second dose (there was a significant correlation, but correlation coefficients <0.7) (Table S4).

Figure 4 shows immunogenicity results in subjects 7–8 months after completing the vaccination schedule. As expected, the specific antibody concentration (median 20.6; 25^{th}–75^{th} percentile 6.9; 58.3) decreased significantly ($p < 0.0001$) compared with those after the second (24.9; 25^{th}–75^{th} percentile 8.2; 85.6) and third doses (121.8; 25^{th}–75^{th} percentile 44.5; 343.7) and with the CCSP (50.8; 25^{th}–75^{th} percentile 23.8; 94.0) (Figure 4A; Table S5). The proportion of subjects with seroconversion after 7–8 months (73.0%) is similar to that obtained after two doses (74.6%) (Table S5). Interestingly, the $mVNT_{50}$ GMT (149.6; 95% CI: 122.3; 182.9) was significantly higher than those after the second dose and the CCSP (Figure 4B; Table S5). At 7–8 months after vaccination, neutralizing antibodies were detected, with $cVNT_{50}$ titers similar to second-dose values (GMT 65.5; 95% CI: 30.5; 140.9) and a reduction of 3.4-fold compared with the third dose (Figure 4C; Table S5).

Safety analysis

Of the 810 participants, 44.4% presented some adverse events (AEs). In total, 947 AEs of 80 types were reported, with 92.6% classified as mild (77.3% consistent

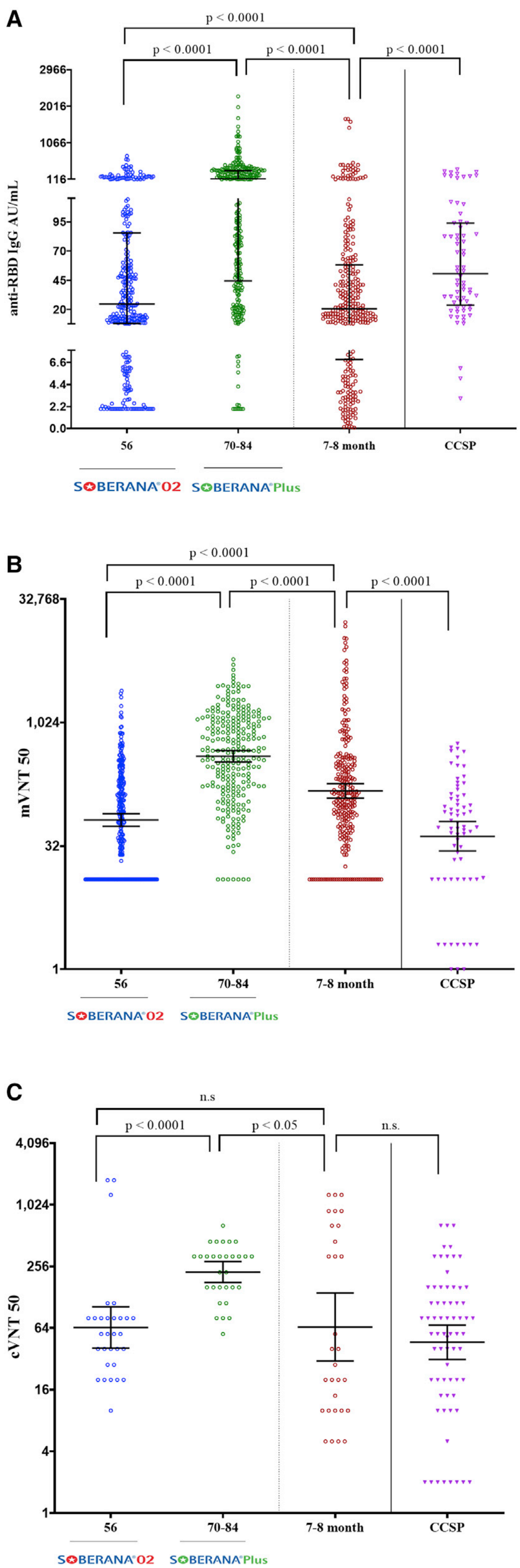
A
p < 0.0001
p < 0.0001
p < 0.0001
p < 0.0001
anti-RBD IgG AU/mL
56
70-84
7-8 month
CCSP
SOBERANA 02
SOBERANA Plus
B
p < 0.0001
p < 0.0001
p < 0.0001
p < 0.0001
mVNT 50
56
70-84
7-8 month
CCSP
SOBERANA 02
SOBERANA Plus
C
n.s
p < 0.0001
p < 0.05
n.s.
cVNT 50
56
70-84
7-8 month
CCSP
SOBERANA 02
SOBERANA Plus

Figure 4. Immunogenicity in vaccinated subjects 7–8 months after completing the immunization schedule (two doses SOBERANA 02 + one dose SOBERANA Plus, 28 days apart)

(A) Anti-RBD IgG concentration expressed in arbitrary units/mL (median, 25^{th}–75^{th} percentile).

(B) Molecular virus neutralization titer $mVNT_{50}$, highest serum dilution inhibiting 50% of RBD:hACE2 interaction (GMT, 95% CI).

(C) Conventional live-virus neutralization titer $cVNT_{50}$ (GMT, CI 95%) against SARS-CoV-2 D614G variant. Blue dots: response at T56, after two doses of SOBERANA 02 of SOBERANA 02 on T0, T28. Green dots: response at T70 or T84 after receiving on T56 the third dose, this one SOBERANA Plus. Brown dots: response after 7–8 months. CCSP (purple), Cuban convalescent serum panel. p value: statistic differences.

with vaccination and 70.7% related to the product under investigation). Eight serious AEs were reported (one—multiform erythema—was consistent with vaccination due to inherent conditions of the subject) (Tables 2 and S6). The most frequent local solicited AE in both vaccine and placebo groups was pain at injection site (35.5% versus 8.8%, respectively) followed by swelling (only in the vaccine group, 13.0%). General discomfort (4.1% versus 2.9% in vaccine and placebo groups, respectively) was the most frequent solicited AE at the systemic level; other AEs had frequencies <1% (Table 3). The frequency of unsolicited AEs was 22.5% and 18.6% in the vaccine and placebo groups, respectively, with headache (5.5%) and hypertension (3.8%) as the most recurrent (Tables S7 and S8). One participant in the vaccine group died from lung and pancreas neoplasm and pneumonia, which were classified as serious and severe AEs but were not consistent with vaccination. The number of vaccinated subjects reporting AEs decreased after the second and third doses (Figure S1; Table S9).

The likelihood ratio (from Bayes factor) was used as the benefit-risk index. Defining benefit as the proportion of individuals with seroconversion at T84 and risk as serious vaccine-associated AE (VAAE), a benefit-risk index of 968 indicates strong evidence for benefit in the vaccine group (Figure S2).

Comparison of two API batches

At day 56, seroconversion was 77.7% (95% CI: 72.9; 82.0) and 74.9% (95% CI: 70.0; 79.4) in the subgroups who received API batch 1 and API batch 2 of SOBERANA 02, respectively. After the third dose, seroconversion increased to 96.5% and 97.0%, respectively, for both batches. A high intersection in confidence intervals was observed for the immunological tests for both batches, suggesting a similar immune response (Tables S10 and S11). The frequency of AEs and their characteristics were similar in both subgroups.

DISCUSSION

This phase IIb trial added further support for the safety of RBD-tetanus toxoid conjugate (SOBERANA 02) and dimeric RBD (SOBERANA Plus) vaccine candidates in a three-dose heterologous scheme, which was already observed in phases I and IIa studies.[6] The proportion of participants with any AE was lower (47.5%) compared with phase I and phase I/II studies for other COVID-19 vaccines produced using several platforms.[8–13] In our study, unlike others,[9,13–15] fever, fatigue, and nausea were not reported or were <1%.

The specific antibody response is relevant for the immune response against SARS-CoV-2.[16,17] The IgG antibody response elicited by vaccination is usually compared with the response induced by natural infection in COVID-19 convalescents.[13,15] Two doses of SOBERANA 02 induced a seroconversion rate of 76.3% and an immune response comparable to the CCSP. The application of a third dose, this one of SOBERANA Plus, increased significantly the number of seroconverted participants to

Table 2. Main characteristics of adverse events following vaccination

	Groups		
	Vaccine (%)	Placebo (%)	Total (%)
N	708	102	810
Subjects with some AEs	336 (47.5)	24 (23.5)	360 (44.4)
Subjects with some VAAEs	311 (43.9)	16 (15.7)	327 (40.4)
Subjects with some serious AEs	4 (0.5)	1 (1.0)	5 (0.6)
Subjects with some serious VAAEs	1 (0.1)	–	1 (0.1)
Subjects with some severe AEs (no VAAEs)	1 (0.1)	–	1 (0.1)
Total AEs	899	48	947
Mild AEs	831 (92.4)	4 (95.8)	877 (92.6)
Moderate AEs	66 (7.3)	2 (2.2)	68 (7.2)
Severe AEs	2 (0.2)	–	2 (0.2)
Serious AEs	7 (0.8)	1 (2.1)	8 (0.8)
Local AEs	583 (64.8)	12 (25.0)	595 (62.8)
Systemic AEs	316 (35.2)	36 (75.0)	352 (37.2)
VAAEs	706 (78.5)	26 (54.2)	732 (77.3)
Serious VAAEs	1 (0.1)	0 (0.0)	1 (0.1)
Severe VAAEs	0 (0.0)	0 (0.0)	0 (0.0)
Reported serious AEs (VAAEs)	multiform erythema	–	–

Vaccine = heterologous scheme (SOBERANA 02 two doses + SOBERANA Plus). Vaccine data correspond to results from participants vaccinated with both API batches. Data are n (%). AE, adverse event; VAAE, vaccine-associated adverse event.

96.8% as well as the concentration of anti-RBD IgG to 122.2 AU/mL. We had reported that a single dose of SOBERANA Plus increased several times the neutralizing IgG antibodies in COVID-19 convalescents;[18] the third dose of SOBERANA Plus had a similar effect in this heterologous schedule, demonstrating the priming effect of the conjugate vaccine in the two-dose regime, inducing an immunological memory as observed in animal models.[4]

The ability of antibodies to inhibit the interaction between recombinant RBD and the human-ACE2 receptor is a proxy for *in vivo* antibody affinity.[19] The virus neutralization titer was comparable to that attained by the CCSP, indicating that the antibodies elicited by the immunogens (a small portion of the viral protein structure) efficiently inhibit virus binding to the ACE2 receptor expressed in Vero cells. All these results are consistent with those obtained in pooled analysis of phase I and IIa clinical trials.[6]

As seen with other viruses, SARS-CoV-2 has evolved, and new variants have been identified, some of which are associated with higher transmissibility and mortality and decreased vaccine efficacy.[20] Epidemiology in Havana showed an evolution in variant predominance in 2021, initially D614G, then Beta (March–June 2021), Delta (July–October 2021), and Omicron (December–ongoing).[21] We found a reduction of $cVNT_{50}$ by 2.4-fold for Delta, 2.5-fold for Omicron, and 7.4-fold for Beta compared with the D614G variant. Similar results have been observed by others: a 3- to 5-fold decrease in neutralizing antibodies against Delta compared with Alpha in vaccinated subjects[22] and, for the Beta variant compared with the original strain, a 7.6- to 9-fold[23] or 10.3- to12.4-fold[24] reduction in neutralization titer was observed in individuals immunized with mRNA vaccines or adenoviral vectors. Interestingly, titers against Omicron variant have a decrease similar to that observed for Delta, whereas another study revealed a 7.1- and 3.6-fold reduction against Omicron compared with D614G and Delta variants, respectively, in subjects vaccinated with heterologous schedule of two doses of CoronaVac and booster with BNT162b2.[25]

Table 3. Characterization of adverse events

	Vaccine (%)	Placebo (%)
N	708	102
Overall AEs within 28 days		
Subject with AE	336 (47.5)	24 (23.5)
Severe (grade 3)	1 (0.1)	0
Serious	4 (0.5)	1 (1.0)
Subjects with solicited AEs		
Any	286 (40.4)	13 (12.7)
Severe (grade 3)	0	0
Serious	0	0
Subjects with solicited systemic AEs		
Any	32 (4.5)	3 (2.9)
General discomfort	29 (4.1)	3 (2.9)
Rash	1 (0.1)	0
Fever	2 (0.3)	1 (1)
Mild fever	1 (0.1)	0
Subjects with solicited local AEs		
Any	294 (38.8)	11 (10.8)
Injection-site pain	251 (35.5)	9 (8.8)
Erythema	13 (1.8)	0 (0.0)
Local warm	43 (6.1)	2 (2.0)
Induration	36 (5.1)	0 (0.0)
Swelling	92 (13.0)	0 (0.0)

Vaccine = heterologous scheme (SOBERANA 02 two doses + SOBERANA Plus). Vaccine data correspond to results from participants vaccinated with both API batches.

Immune response can be influenced by several factors like age, presence of comorbidities, and sex.[26–28] Here, vaccination induced a significant increase in all immunological variables in each analyzed subgroup (male and female, 19–59 and 60–80 years old, subjects with and without comorbidities) compared with placebo. However, a significantly higher response was observed in age subgroup 19–59 and in participants without comorbidities. As noted with other anti-SARS-CoV-2 vaccines, elders elicited lower titers of specific IgG and neutralizing antibodies when compared with younger subjects (an approximately 2-fold reduction in anti-RBD IgG and ACE2 competition after 2 doses with mRNA vaccines[29] or 1.8- to 2.96-fold decrease, depending on the dose-in neutralizing antibodies after immunization with AS03-adjuvanted recombinant protein vaccine[30]). In our previous phase IIa clinical trial, no differences between both age subgroups, except for $mVNT_{50}$, were noted.[6] This may be related to the smaller number of elderly subjects included in phase IIa (24) compared with the 157 elders in phase IIb. Concerning comorbidities, Güzel et al. also found a negative relationship between immune response and the presence of diabetes mellitus and cardiovascular disease.[31]

Durability of immune response for anti-COVID-19 vaccines is an issue of utmost importance. In this work, concentration of anti-RBD IgG after 7–8 months decreased 5.9- and 2.44-fold compared with post-third dose and with CCSP values, respectively. Also, molecular and viral neutralization titers decreased with respect to the value after the third dose (2.65- and 3.4-fold reduction, respectively), but high levels of neutralizing antibodies were still detected after 7–8 months of vaccination. Levin et al. observed an 18.3-fold waning of antibody titers in subjects after 6 months of two doses of BNT162b2 vaccine, whereas a much lower decrease (4.66-fold) was detected in GMTs of neutralizing antibody.[32]

Cellular immune response elicited by this heterologous vaccine combination is characterized by a Th1/Th2 mixed profile, as we previously reported during phase IIa.[6] Despite the fact that T cell response was not studied during this phase IIb, the persistence of neutralizing antibodies as a probe of long-lasting immunity could be related to an efficient cooperation of T cell responses during the priming. Further evaluation of $CD8^+$ and $CD4^+$ T cell populations will give more elements about the T cell response induced by vaccination with this heterologous combination.

A prediction of clinical efficacy has been reported for seven vaccines based on immunogenicity data.[33,34] We used our cumulative data for IgG antibodies and $cVNT_{50}$ against D614G variant from phases I, IIa, and IIb (for SOBERANA 02, 25 μg; two-dose and heterologous three-dose schedules) to estimate the efficacy, using the same ratio of vaccinees versus CCSP. The efficacy for the two-dose schedule was estimated to be between 58% and 87% and for the three-dose scheme between 79% and 93% (Figure S3). These results have been confirmed in a phase III clinical trial conducted in Havana during March–July 2021. The preliminary report calculated 71% of efficacy for the two-dose schedule of SOBERANA 02 and 92.4% for the heterologous three-dose schedule.[35]

In conclusion, two doses of SOBERANA 02 or SOBERANA 02 + SOBERANA Plus combined in a heterologous schedule were immunogenic, well tolerated and safe in adults aged 19–80 years. The third dose of SOBERANA Plus increased significantly the neutralizing antibody titers. Results obtained here confirmed phase I and IIa results and paved the way for phase III clinical evaluation.

Limitations of the study

Participants during the follow-up period (7–8 months after the third dose) were followed for the presence of any COVID-19 symptom. Those suspected as possibly infected were excluded from the subgroup analyzed at 7–8 months; nevertheless, potential infections were not investigated through PCR or qualitative rapid antigen test. In consequence, asymptomatic COVID-19 cases could not be excluded.

Even when subjects with comorbidities were included in this trial (bronchial asthma, ischemic heart disease, hypertension, pituitary adenoma, prostatic adenoma, diabetes mellitus, chronic obstructive pulmonary disease, prostatic hyperplasia, Parkinson's disease), no particular analyses were made in order to elucidate which comorbidity has more impact on immunogenicity. This could be a goal for further studies. The same could be applicable for special population like pregnant or breast-feeding women and individuals with low or high BMI.

CONSORTIA

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STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2022.08.001.

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AUTHOR CONTRIBUTIONS

V.V.-B., D.G.-R., Y.V.-B., S.F.-C., Y.C.-R., D.S.-M., D.G.R., T.B.-A., G.-W.-C., and B.S.-R. conceived the vaccine candidate. M.E.T.-R., C.V.-S., V.V.-B., D.G.-R., and Y.V.-B. conceived the study protocol and were involved in data analysis and interpretation. D.D. and A.B. participated in designing of protocol study. M.G.-C., L.V.-S., S.P.-R., Y.G.-M., R.G.-M., and B.L.S.-V. were responsible for the site work, including the recruitment and data collection. M.R.-G., B.P.-M., I.M.-H., and Y.M. supervised and monitored the trial. B.S.-R., T.H.-G., I.O.-V., M.D.-H., M.T.P.-G., J.E.-P., E.N.-R., A.P.-D., G.B.-R., and L.R.-N. carried out immunological experiments and the analysis of results. C.V.-S. was involved in data curation and statistical analysis of data. G.-W.-C. supplied resources. S.F.-C., Y.G.-V., C.V.-S., D.G.-R., M.E.T.-R., and V.V.-B. wrote the manuscript. M.E.T.-R., C.V.-S., and R.G.-M. had unrestricted access to all data. All authors agreed to submit the manuscript, read and approved the final draft, and take full responsibility of its content, including the accuracy of the data and the fidelity of the trial to the registered protocol and its statistical analysis.

DECLARATION OF INTERESTS

M.E.T.-R., M.G.-C., L.V.-S., S.P.-R., C.V.-S., M.T.P.-G., J.E.-P., E.N.-R., A.P.-D., G.B.-R., I.M.-H., Y.M., Y.G.-M., B.L.S.-V., G.-W.-C., D.D., A.B., and D.G.R. declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. M.R.-G., B.P.-M., B.S.-R., R.G.-M., T.H.-G., I.O.-V., M.D.-H., S.F.-C., Y.C.-R., L.R.-N., D.S.-M., Y.G.-V., T.B.-A., Y.V.-B., D.G.-R., and V.V.-B. work at Finlay Vaccine Institute or the Center of Molecular Immunology, institutions that develop and manufacture the vaccine candidates, but they have not received an honorarium for this paper. B.S.-R., S.F.-C., Y.C.-R., L.R.-N., D.S.-M., Y.V.-B., D.G.R., D.G.-R., and V.V.-B. have filed patent applications related to the vaccine SOBERANA 02.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Monoclonal Anti-Human IgG (Fc specific)-Biotin antibody produced in mouse	Sigma Aldrich	Cat# B3773, RRID:AB_258559
Anti-Mouse IgG (whole molecule)-Alkaline Phosphatase antibody produced in goat	Sigma Aldrich	Cat# A9316, RRID:AB_258446
Bacterial and virus strains		
hCoV-19/Cuba/DC01/2020	Cuban Collection at the National Civil Defence Research Laboratory	EPI_ISL_7495115|2020-06-05
hCoV-19/Cuba/DC03/2020	Cuban Collection at the National Civil Defence Research Laboratory	EPI_ISL_7495130|2020-12-24
hCoV-19/Cuba/DC07/2021	Cuban Collection at the National Civil Defence Research Laboratory	EPI_ISL_7495144|2021-04-20
hCoV-19/Cuba/DC05/2021	Cuban Collection at the National Civil Defence Research Laboratory	EPI_ISL_7495138|2021-04-20
hCoV-19/Cuba/DC-RRR/2021	Cuban Collection at the National Civil Defence Research Laboratory	EPI_ISL_12691753|2022-05-15
Biological samples		
Human serum samples obtained from voluntaries	This paper	N/A
Cuban convalescent serum panel	Toledo-Romani et al.[6]; Chang-Monteagudo et al.[18]	N/A
Chemicals, peptides, and recombinant proteins		
Streptavidin/alkaline-phosphatase	Roche, Basel, Swiss	Cat#10556602103
4-methylumbelliferyl phosphate	SLS	Cat#M3168
hFc-ACE2 protein	Center for Molecular Immunology, Cuba Toledo-Romani et al.[6]; Chang-Monteagudo et al.[18]	N/A
Recombinant RBD-mouse-Fc	Center for Molecular Immunology, Cuba Toledo-Romani et al.[6]; Chang-Monteagudo et al.[18]	N/A
Critical commercial assays		
UMELISA SARS-CoV-2 anti- RBD kit	Center for Immunoassay, Havana, Cuba[36]; Tan et al.[37]	UM 2045/2145
Experimental models: Cell lines		
Vero E6	ATCC	Cat# CRL-1586, RRID:CVCL_0574
Software and algorithms		
Prism 6	GraphPad	https://www.graphpad.com
SPSS 25.0	IBM	https://www.ibm.com/analytics/spss-statistics-software
EPIDAT 12.0	SERGAS	https://www.sergas.es/
Ultramicroanalytic (SUMA) software	Center for Immunoassay, Havana, Cuba	SRS v9.29

RESOURCE AVAILABILITY

Lead contact

Additional information and requests for resources and reagents should be directed to the lead contact, Sonsire Fernández-Castillo (sfernandez@finlay.edu.cu).

Material availability

This study did not generate new reagents.

Data and code availability

All data reported in this paper will be shared by the lead contact upon request.
This study did not generate any new codes.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethical considerations

The phase II clinical trial protocol was reviewed and approved by an *ad hoc* centralized Research Ethics 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). The Cuban National Regulatory Agency (CECMED) approved the trial and the procedures (reference number: 05.019.20BA, 17th December 2020).

The National Clinical Trials Coordinating Center (CENCEC) was responsible for monitoring data accuracy, adherence to the protocol and to Good Clinical Practice. An Independent Data Monitoring Committee (conformed by six external and independent members specialized on clinical practice, epidemiology and statistic) supervised the study.

The trial was conducted following the Declaration of Helsinki, Good Clinical Practice and the rules of the Cuban National Immunization Program. During participants recruitment, the potential participants received all relevant information (both orally and written) about the vaccine candidates, and the potential risks and benefits of the trial. All doubts were clarified before enrollment. The decision to participate in the study was voluntary and not remunerated. Written informed consent was obtained from all participants.

The characteristics of the participants in this study are summarized in Table 1. Information on socioeconomic status was not collected.

METHOD DETAILS

Participants and study design

Phase IIb was designed as a multicenter, adaptive, parallel, double blind, randomized, placebo-controlled trial for evaluating the immunogenicity, safety and reactogenicity of two doses of SOBERANA 02 and the heterologous scheme with a third dose with SOBERANA Plus. Healthy adults aged 19-80 years, of both sexes were recruited through public advertisement at community or professional environment close to the clinical site. Detailed information about all eligibility criteria are summarized in International Clinical Trials Registry Platform 7.

Two production batches of active pharmaceutical ingredient (API) of SOBERANA 02 were evaluated. Participants were randomly assigned at a 4:4:1 ratio to receive one of the two API batches of SOBERANA 02 or placebo (810 subjects; API 1: 354, API 2: 354 and 102 in the placebo group). Randomization was stratified in four 10-years age subgroups (from 19-29, 30-39, 40-49, 50-59 years), and one 21-years age subgroup (60-80 years).

The trial was conducted at two clinical sites: Clinic #1 at "La Lisa" Municipality and Polyclinic "19 de Abril" at "Plaza de la Revoluci'n" Municipality, Havana, Cuba. (Cuban Public Registry of Clinical Trials, included in WHO International Clinical Registry Trials Platform: https://rpcec.sld.cu/trials/RPCEC00000347.[7])

Products under evaluation

SOBERANA 02 (FINLAY-FR-2) and SOBERANA Plus (FINLAY-FR-1A) are vaccine candidates based on the recombinant receptor binding domain (RBD, strain D614G) of

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SARS-CoV-2 virus produced in CHO cells. The RBD sequence, Arg319-Phe541-(His) 6, includes free Cys538, a suitable conjugation site to tetanus toxoid (in SOBERANA 02),4 and allowing RBD dimerization (in SOBERANA Plus).3 Vaccines and placebo were produced under Good Manufacturing Practice at Finlay Vaccine Institute and the Center of Molecular Immunology in Havana, Cuba. Two SOBERANA 02 API batches resulted in three final product batches: EC-CVRBDC-2003 and EC-CVRBDC-2004 (API 1), and EC-CVRBDC-2005 (API 2); SOBERANA Plus batches were EC-CVRBDd-2008 and EC-CVRBDd-2101; placebo (only excipients of SOBERANA 02) batch was: E1001PS02. Vaccines and placebo composition were described below. Vaccine and placebo formulations were visually undistinguishable.

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

Procedures

Participants received intramuscular injections in the deltoid region, 28 days apart. They were closely followed for one hour after each injection for safety evaluation. Medical visits were planned at 24, 48, and 72 h, 14 and 28 days after each dose. Adverse events were self-registered by the participants on a diary card and recorder during medical visits.

Serum samples were collected on days 0 (baseline) and 56 from all subjects; on days 14 and 70, blood samples were taken from 50% of the participants while samples from the other 50% were collected on days 42 and 84. For that, at the beginning of the trial, a simple random sampling was performed to assign 50% of subjects in each subgroup.

To evaluate the persistence of the humoral response, 7-8 months after completing the vaccination schedule another serum sample was obtained from a subset of vaccinated participants.

Outcomes

The primary outcomes were percentage of subjects with seroconversion ≥4-fold the anti-RBD IgG pre-vaccination level. Secondary outcomes included: 1) Serious Adverse Events (AEs) measured daily for 28 days after each dose; 2) Solicited Local and Systemic AEs for 7 days after each dose; 3) Unsolicited AEs measured daily for 28 days after each dose; 4) Conventional neutralizing antibody titers ($cVNT_{50}$) of a subset of samples from seroconverted subjects and 5) Inhibition of RBD-hACE2 interaction expressed as % and molecular inhibitory titer ($mVNT_{50}$). Outcomes are detailed in International Clinical Trials Registry Platform.[7]

Immune response assessment

All immunological evaluations were performed by external laboratories on blind samples.

Anti-RBD IgG concentration, inhibition of RBD-hACE2 interaction and $mVNT_{50}$ were determined on days 0, 14, 42, 56, 70, 84. From the subjects with seroconversion, around a 10% were selected using simple random sampling for conventional neutralizing antibody titers ($cVNT_{50}$) against D614G variant on days 0, 56, 70 and 84. $cVNT_{50}$ against VOC was also determined in a subset of samples with $cVNT_{50}$ vs. D614G >20. Molecular neutralization assay (% Inhibition RBD:hACE2) was determined at T0 only if the sample has pre-vaccination IgG concentration over 7.8 AU/ml (4-fold the limit of quantification in ELISA assay, 1.95 AU/ml). Anti-RBD IgG concentration, $mVNT_{50}$ and $cVNT_{50}$ were also determined after 7-8 months of the last dose.

The humoral immune response was compared with that of a Cuban Convalescent Serum Panel (CCSP) made with serum from 68 COVID-19 convalescent patients and characterized with the same techniques used in clinical trials.[6,18] Serum samples were taken 2-4 months after infection with SARS-CoV-2 virus.

Anti-RBD IgG response. Anti-RBD IgG in sera was evaluated by a quantitative ultramicro ELISA (UMELISA SARS-CoV-2 anti- RBD, Center for Immunoassay, Havana, Cuba) using d-RBD as coating antigen (4 μg/mL) and an in-house standard- characterized serum, which was arbitrarily assigned 200 AU/mL (based on a half-maximal inhibitory titer of 200 and a conventional virus neutralization titer of 160). The standard curve comprised six two-fold serial dilutions (0, 4, 8, 16, 32 and 64 AU/mL) of the standard. Samples were evaluated in duplicate. After incubation step, biotin-conjugate anti-IgG human (0.1 μg/mL) (Sigma Aldrich, San Luis, EE UU) and later, streptavidin/alkaline-phosphatase Roche, Basel, Swiss) in appropriate buffers were added. The final fluorimetric reaction was induced by adding the substrate 4-methylumbelliferyl phosphate (SLS). The reference curve was constructed using a linear interpolation function. 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. Serum samples for this assay were extracted on days 0, 14, 42, 56, 70, 84 and 7-8 months after the last dose

Molecular viral neutralization test. This ELISA is an *in-vitro* surrogate of the life-virus neutralization.[37] It uses recombinant RBD-mouse-Fc (RBD-Fcm) and the host cell receptor hACE2-Fc (ACE2-Fch) as coating antigen. Human antibodies against RBD can block the RBD-Fcm interaction with ACE2-Fch. The RBD-Fcm that was not inhibited can bind to ACE2-Fch, and is recognized by a monoclonal antibody anti-γ mouse conjugated to alkaline phosphatase. The results are expressed as % inhibition of RBD-hACE2 interaction (at a serum dilution of 1/100); and as the half molecular virus neutralization titre ($mVNT_{50}$) represented as the maximal serum dilution inhibiting 50% of RBD-hACE2 interaction. Serum samples for this assay were extracted on days 0, 14, 42, 56, 70, 84 and 7-8 months after the last dose. Molecular neutralization assay was determined at T0 only if the sample has pre-vaccination IgG concentration over 7.8 AU/ml (4-fold the limit of quantification in ELISA assay, 1.95 AU/ml).

Conventional viral neutralization test. Neutralizing antibodies against live SARS-CoV-2 was performed in a biosecurity laboratory level 3 (National Civil Defence Research Laboratory, Havana, Cuba) by the conventional virus neutralization test, the gold standard for determining antibody efficacy against SARS-CoV-2, following the recommendation of Manenti & cols.[38] Serial dilutions of heat-inactivated serum samples (starting from 1:5) in Eagle's Minimal Essential Medium (Gibco, UK) containing 2 % fetal bovine serum (Capricorn, Germany) were incubated for 1 hour at 37°C with an equal volume of viral solution containing 100 $TCID_{50}$ of SARS-CoV-2 strains: CU2010-2025, variant D614G (hCoV-19/Cuba/DC01/2020/ GIDAID: EPI_ISL_7495115|2020-06-05); CU2101-2102, variant B.1.1.7 alpha (hCoV-19/Cuba/DC03/2020/ GIDAID: EPI_ISL_7495130|2020-12-24); CU2104-2179, variant B.1.617.2 delta (hCoV-19/Cuba/DC05/2021: GIDAID: EPI_ISL_7495138|2021-04-20); CU2104-2180, variant B.1.351 beta (hCoV-19/Cuba/DC07/2021/ GISAID: EPI_ISL_7495144|2021-04-20); RRR, variant BA1.21K omicron (hCoV-19/Cuba/DC-RRR/2201/ GISAID: EPI_ISL_12691753|2022-05-15); Cuban Collection at the National Civil Defence Research Laboratory) in cell plates containing a semi-confluent Vero E6 monolayer (10^4 cell/well). The highest serum dilution showing an OD at 540 nm, representing the 50% of average OD values from control cell wells (Vero E6 monolayer with mixture of virus-serum) was considered as the neutralization titer and is represented as neutralizing titer 50 ($cVNT_{50}$). Conventional neutralizing antibody titers ($cVNT_{50}$) against D614G variant were evaluated in a subset of samples randomly selected from the individuals with seroconversion on days 0, 56, 70 and 84. $cVNT_{50}$ against VOC was also determined in a subset of samples with $cVNT_{50}$ vs. D614G >20.

Safety evaluation

Solicited local and systemic AEs were measured daily from days 0 to 7 following each immunization. Other AEs were self-recorded until completion of the 28 days follow-up period. The severity of solicited AEs was graded according to Brighton Collaboration definition and the Common Terminology Criteria for Adverse Events version 5·0. All AEs were reviewed for causality and classified according to WHO.[39]

Statistical analysis

Calculation of sample size was done before starting phase II study (this included phases IIa and IIb) considering a two-sided 95% confidence interval for the difference between two proportions with a width of 0.16, to estimate a difference between each API batch and placebo group of around 50%, with a lower bound of the confidence interval > 30% and a dropout of 15%. This resulted in a phase II sample size of 910 subjects randomized 4:4:1 in three groups (vaccine API 1, vaccine API 2 and placebo) (404:404:102), and allowing a loss of up to 138 subjects. Stage IIb excluded the 100 participants in phase IIa, giving a sample size of 810 subjects. The evaluation of the study hypothesis remaining valid after excluding stage IIa participants.

Safety and reactogenicity endpoints are described as frequencies (%). Quantitative demographic characteristics are reported as mean, standard deviation (SD), median, interquartile range, and range. We calculated seroconversion rate for anti-RBD IgG antibodies ($\geq$4-fold increase in antibody concentration over baseline) for each subject. Anti-RBD IgG concentration and % of inhibition of RBD-hACE2 interaction were expressed as median and interquartile range; molecular virus neutralization titer ($mVNT_{50}$) and conventional virus neutralization titer ($cVNT_{50}$) were expressed 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 t-Test or the Wilcoxon Signed-Rank Test were used for before-after statistical comparison.

Immunogenicity were performed in the "full analysis set" (FAS, all subjects randomly assigned to a treatment group having at least one efficacy assessment after randomization) and safety was analyzed in the "safety set" (all subjects who received at least one dose).

Statistical analyses were done using SPSS version 25.0; EPIDAT version 12.0 and Prism GraphPad version 6.0. An alpha signification level of 0.05 was used.