An open-label phase IIa and double-blind, randomised, placebo-controlled phase IIb seamless clinical trial of FINLAY-FR-1A vaccine: safety and immunogenicity in COVID-19 convalescents

Abstract

Background

A phase I clinical trial to evaluate FINLAY-FR-1A vaccine in COVID-19 convalescents was completed. Here, we report results of the phase II clinical trial.

Methods

We studied 450 convalescents with history of asymptomatic, mild or moderate COVID-19. Phase II was sequentially performed in two stages: 1) open, non-controlled phase IIa in subjects aged 60-78 years (N=20), and 2) placebo-controlled and double-blind phase IIb trial in subjects aged 19-78 years, randomised into two groups: experimental (N=344), vaccinated with a single dose of the FINLAY-FR-1A vaccine (50 µg of recombinant dimeric-RBD) and control (placebo) (N=86). The primary outcomes were safety, evaluated 28 days after vaccination by the occurrence of serious adverse events, and successful immune response, assessed by neutralizing antibody ELISA, and defined as half-maximal surrogate virus neutralization titres ≥250. Vaccine immunogenicity at baseline and after vaccination was also assessed by ELISA anti-RBD and live-virus neutralization test. Cuban Public Registry of Clinical Trials, WHO-ICTRP: https://rpcec.sld.cu/en/trials/RPCEC00000366-En.

Findings

No vaccine-associated serious adverse events were reported. Minor adverse events were found, the most common, local pain: 105 (29%). A successful immune response was found in 81% of subjects. The vaccine elicited a >31-fold increase in anti-RBD-IgG antibodies, and the seroconversion rate was 84% on day 28 after vaccination; the geometric mean titres of live-virus neutralization test increased up to 400·3 and high response was found against Alpha, Beta and Delta variants of concern.

Interpretation

A single dose of the FINLAY-FR-1A vaccine against SARS-CoV-2 strengthened the pre-existing natural immunity, with excellent safety profile.

Funding

Cuba's Ministry of Science, Technology and Environment.

Research in context

Evidence before this study

Immunity against SARS-CoV-2 is highly dependent on the level and quality of neutralizing antibodies, though the T-cell response plays an important role in COVID-19 mitigation. Persons recovered may be reinfected, particularly those with low neutralizing antibody titres and facing new SARS-CoV-2 variants of concern. Severe SARS-CoV-2 reinfections with Delta variant have been reported, and evidence suggests an increased risk of reinfection with new Omicron variant. A phase I clinical trial of FINLAY-FR-1A vaccine conducted in COVID-19 convalescents demonstrated that SARS-CoV-2 infection induces long-term memory immune cells that are activated by a single vaccine dose. Pubmed (https://pubmed.ncbi.nlm.nih.gov) was searched using the terms: "Clinical trial" [Publication Type] AND "COVID-19 vaccines" [MeSH Terms] OR "SARS-CoV-2" [Text Word] OR "COVID-19" [Text Word] AND convalescent [Text Word] OR infected [Text Word] OR recovered [Text Word]. The only restriction was language (English) and no time limit was planned. Only four post-licensing studies of COVID-19 vaccines in previously infected subjects were recovered, all involving a small number of subjects. MedRxiv (https://www.medrxiv.org) (subject area: infectious diseases) was also searched (search terms as described for Pubmed): three additional trials were recovered (seven, in total), all studies with a different design than our clinical trial and reporting a secondary antibody response induced by vaccination.

Added value of this study

This is a randomised, placebo-controlled phase II clinical trial of an anti-SARS-CoV-2 vaccine, especially designed for COVID-19 convalescents. The vaccine demonstrated to be safe with good tolerability, evidenced by the fact that most local and systemic reactions were mild. RBD:hACE2 binding inhibitory antibodies were induced in most volunteers after a single vaccine dose, which prove its immunogenicity. There was also an increase in live-virus neutralizing titres against the Alpha, Beta and Delta variants of concern. Results confirm that natural infection leads to the production of long-term memory B cells that respond quickly to a single dose of FINLAY-FR-1A vaccine.

Implications of all the available evidence

An RBD vaccine can be used to trigger immunity against SARS-CoV-2 in COVID-19 convalescent individuals, including those with low levels of neutralizing antibodies. Immunization with a single dose of FINLAY-FR-1A vaccine triggered a rapid induction of high humoral immune response, suggesting a protective immunity against SARS-CoV-2, and suggesting a decrease in severe reinfection by SARS-CoV-2 variants of concern.

Introduction

The number of persons recovered from COVID-19 is increasing. By the end of 2021, from about 300 million cases reported worldwide, the number of individuals recovered from SARS-CoV-2 infection is surpassing 250 million.[1]

The efficiency and duration of protection elicited by viral infection is not well known, but they probably depend on the quality and intensity of the specific immune response.[2-6] On the other hand, there is evidence of reinfection especially after emerging of variants of concern (VOCs). Severe SARS-CoV-2 reinfections with Delta variant have been reported after recovery from COVID-19,[7-10] and evidence suggests an increased risk of reinfection with new Omicron VOCs.[10]

Vaccine candidates based on the receptor-binding domain (RBD) developed on different platforms, have demonstrated safety and immunogenicity.[11-13] FINLAY-FR-1A (SOBERANA Plus) vaccine is based on a recombinant protein antigen, a dimer of RBD with sequence 319-541 obtained in genetically modified Chinese hamster ovary cells (CHO). RBD is dimerized (d-RBD) through a Cys538–Cys538 interchain disulphide bridge.

The antigen is adsorbed on aluminium hydroxide gel, and it is produced under Good Manufacturing Practice at The Finlay Vaccine Institute and The Centre of Molecular Immunology, in Havana, Cuba. It was evaluated in a phase I clinical trial in naïve individuals and in a phase I trial carried out in COVID-19 convalescents.[14,15]

Convalescent subjects of mild COVID-19 and individuals with subclinical infection received a single intramuscular injection of the FINLAY-FR-1A (SOBERANA Plus, 50 μg). The vaccine was safe; minor adverse events were only found. A high humoral and cellular immune response were detected. Live-virus neutralization titres higher than 160 were found in 80% of participants. Also, the correlation between the live-virus neutralization test and in-vitro techniques was demonstrated, especially with the half-maximal surrogate virus neutralization titres.[15]

There is evidence that natural infection leads to the production of long-term memory cells that can respond quickly to a single dose of FINLAY-FR-1A (SOBERANA Plus) vaccine.[15] Here, we study in depth the humoral immune response to assess the response of memory B cells after a single dose of the vaccine in individuals with past SARS-CoV-2 infection.

Methods

Study design and participants

This phase IIa/IIb clinical trial was carried out at the National Institute of Haematology and Immunology and the National Centre for Sexual Education (as vaccination facilities), both located in Havana, Cuba. Four hundred and fifty convalescents of both sexes aged 19-78 years with history of asymptomatic, mild or moderate COVID-19 were recruited in Havana, Cuba, among COVID-19 convalescents who fulfilled the selection criteria (Supplementary material, Appendix 1, Appendix 3).

Due to safety concern, and in accordance with the requirements of the Cuban protocol for convalescent patients,[16] COVID-19 convalescents had been discharged from hospitals at least two months before beginning the study. The time elapsed from hospital discharge to vaccination was computed (according to Cuban regulations, all individuals with positive-PCR tests, including those asymptomatic, were admitted to hospitals). A negative PCR test at least two months before the initiation of the study was required.

Participants were randomly assigned to experimental or control groups: the experimental group was vaccinated with a single dose of FINLAY-FR-1A (SOBERANA Plus) vaccine, the control (placebo) group received vaccine excipient. Adverse events and the humoral immune response were evaluated as will be described in “Procedures”.

A two-stage seamless trial design was performed. Phase IIa: open, non-controlled stage with a single experimental group in adults aged 60-78 years. Participants of this age subgroup would be included in phase IIb if the vaccine-associated serious adverse events rate was lower than 0·05, and the probability of achieving a successful immune response (defined in “Procedures”) >50% was not less than 0·1. Phase IIb: randomised, placebo-controlled, and double-blind stage. Based on phase IIa results, phase IIb included convalescent subjects aged 19-78 years, randomised into two groups: the experimental group receiving the intervention, and the control group.

All participants underwent a screening visit (full medical history, rapid pregnancy test in women of childbearing potential, SARS-CoV-2 rapid antigen test (Roche). Full blood count, kidney and liver

function tests were done only in phase IIa). Exclusion criteria were: history of severe COVID-19, hospitalization due to COVID-19 during the last two months, any severe disease or decompensated chronic disease, immunodeficiency, history of severe allergy, pregnancy, breastfeeding, positive SARS-CoV-2 test, immunological treatment during the last 30 days, history of having received any vaccine against SARS-CoV-2 (Supplementary material, Appendix 3). The study was registered at the Cuban Public Registry of Clinical Trials: https://rpcec.sld.cu/en/trials/RPCEC00000366-En, included in WHO International Clinical Registry Trials Platform.

Ethical considerations

The Cuban Ministry of Public Health established a medical care program for COVID-19 convalescent patients,[16] and approved the trial and the procedures. The National Institute of Haematology and Immunology —main clinical site of the trial—, the National Centre for Sexual Education —secondary clinical site— the Independent Ethics Committee for Studies on Human Subjects, and the Cuban National Regulatory Agency (Centre for State Control of Medicines and Medical Devices, CECMED), approved the trial and the procedures (CECMED, Authorization date: April 9, 2021, Reference number: 110/05·008·21BA). It was conducted according to the Declaration of Helsinki and Good Clinical Practice.

The clinical trial was monitored by the National Coordinating Centre of Clinical Trials. In addition, an Independent Data Monitoring Committee specialized in clinical trials and data monitoring, independent from sponsors and clinical investigators, performed an interim data analysis of safety, reactogenicity and early immunogenicity on day 14 post-vaccination in the phase IIa. It provided supervision during all the trial. The final analysis of safety, reactogenicity, and immunogenicity in phases IIa and phase IIb were done by the statistician responsible of the design and statistical analysis. All subjects were studied on day 14 (interim analysis, phase IIa), and on day 28 for final analysis (both trial phases).

During recruitment, investigators provided potential participants with extensive oral and written information. All questions and doubts were clarified. The decision to participate in the study was completely voluntary and non-remunerated. Written informed consent was obtained from all participants. During the study, the Committees assessed the trial's risk-benefit ratio and assured the rights, health and privacy of volunteers, including information confidentiality.

Product under evaluation

Vaccine antigen: SARS-CoV-2 RBD (sequence: 319-541 amino acid residues with a poly-histidine fusion tag at its C-terminus), expressed in CHO cells. RBD is dimerized through a Cys538–Cys538 interchain disulphide bridge. FINLAY-FR-1A (SOBERANA Plus) vaccine, composition per dose (0·5 mL): d-RBD 50 µg, NaCl 4·250 mg, Na_2HPO_4 0·03 mg, NaH_2PO_4 0·02 mg, thiomersal 0·05 mg, injection water, aluminium hydroxide gel 1·25 mg, pH 6·0–7·2. The control group was injected with vaccine excipient. Vaccine and placebo were manufactured according to Good Manufacturing Practice by the Finlay Vaccine Institute and the Centre of Molecular Immunology in Havana, Cuba.

Randomisation and blinding

After medical screening of volunteers with history of COVID-19, 450 eligible subjects between 19-78 years old were recruited. Sample size was calculated as will be described in "Statistical analysis". Twenty subjects aged 60-78 years were included in the open, single-group, phase IIa. They were randomly selected among this age subgroup in the recruited population. In phase IIb, 430 participants were randomly allocated 4:1 to two groups: experimental (vaccine) and control (placebo). Stratified random blinded sampling proportionally divided participants in two age subgroups: 19-59 and 60-78 years to ensure a representation of each age subgroup according to national reports of COVID-19 age incidence. Allocation of participants in each group was done by simple random blinded sampling using a centralized technology. Each participant got an identification code, which matched the vial label code. Study participants were enrolled by the research team. The research product management specialist generated the random allocation sequence and assigned participants to interventions.

All study staff, investigators, sponsors and subjects, remained blinded until the conclusion of the study (28 days after the vaccine was applied to all volunteers). All vials had the same characteristics: R2 vial, single dose, volume and pink cap.

Procedures

All participants received a single deltoid intramuscular injection (0·5 mL) of the vaccine or placebo. Volunteers were closely observed for one-hour post-vaccination. After vaccination, active surveillance by health care professionals was carried out on days 1, 2, 3, 7 and 28, plus day 14 in phase IIa. Participants were instructed to complete a diary record of solicited local and systemic adverse reactions during the 28 days follow-up period.

Solicited and protocol-defined local site reactions (injection site pain, warmth, redness, swelling, induration) and systemic symptoms (general malaise, rash, and fever defined as an axillary

temperature ≥38°C) were recorded for seven days. All other events were recorded throughout the 28 days follow-up period. The intensity of expected and protocol-defined local and systemic adverse events were graded as mild, moderate and severe, according to Brighton Collaboration definition and the Common Terminology Criteria for Adverse Events version 5·0. Intensity of unsolicited adverse events were graded as mild (transient or mild discomfort, no interference with activity), moderate (mild to moderate limitation in activity), or severe (marked limitation in activity).[17,18] All adverse events were reviewed for causality, and events were classified according to WHO: Inconsistent causal association to immunization, consistent causal association to immunization, indeterminate, unclassifiable.[19]

Blood samples were taken on days 0 (before vaccination), 14 and 28 in phase IIa, and on days 0 and 28 in phase IIb.

Humoral immune response at baseline and following vaccination was evaluated by:

a) *UMELISA SARS-CoV-2 ANTI-RBD*. This is a commercial (Immunoassay Centre, Havana, Cuba) quantitative IgG anti-RBD ultra-micro ELISA, based on d-RBD as coating antigen and streptavidin-biotin technology (biotin-conjugated anti-human-γ, streptavidin-alkaline phosphatase conjugate and 4-methylumbelliferyl phosphate as fluorometric substrate). A standard curve from 0 to 64 U/mL is used for the quantitative determination of IgG anti-RBD. The IgG anti-RBD concentration was determined by interpolating the fluorescence of serum samples in the standard curve constructed using the ultra-microanalytic (SUMA) software.[20] Seroconversion rates for IgG anti-RBD antibodies (≥4-fold increase in antibody titres over pre-immunization titres) were calculated for all subjects.

b) *SARS-CoV-2 neutralizing antibody ELISA*. It is based on antibody-mediated blockage of RBD:hACE2 interaction, and can be considered as an *in-vitro* surrogate of the live-virus neutralization test. 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 interaction of RBD-Fcm with ACE2-Fch. The RBD-Fcm that was not inhibited can bind to ACE2-Fch, and it is recognized by a monoclonal antibody anti-γ murine conjugated to alkaline phosphatase. The inhibition ratio of RBD:hACE2 interaction at a serum dilution of 1/100 and the half-maximal surrogate virus neutralization titres ($sVNT_{50}$) were calculated; $sVNT_{50}$ is the serum dilution inhibiting 50% of RBD:hACE2 interaction.[15,21] A successful immune response was considered if $sVNT_{50}$ ≥250; a value six times higher than the geometric mean of $sVNT_{50}$ of the Cuban Convalescent Serum Panel (CCSP) and four times higher than the upper limit of the 95% confidence

interval, and correlating with live-virus neutralization titres above 80.[15] All subjects were evaluated with this neutralizing antibody ELISA.

c) *Conventional live-virus neutralization test*. This assay is the gold standard for determining antibody efficacy against SARS-CoV-2. It is a colorimetric assay based on antibody neutralization of SARS-CoV-2 cytopathic effect on Vero E6 cells.[15,21] The viral neutralization titres (cVNT) against the D614G variant were assessed in all phase IIa subjects and a subsample of 10% in phase IIb, randomly selected from participants with a successful immune response. Among them, ten samples were selected by simple random sampling and evaluated against Alpha, Beta, and Delta VOCs in the Hospital "Amedeo di Savoia", Turin, Italy.

The vaccine-elicited humoral immune response was compared with that of the CCSP, composed of 68 serum samples from asymptomatic individuals (25), and those recovered from mild/moderate (30) and serious (13) COVID-19. This panel was previously characterized by ELISA, in-vitro inhibitory assay and live-virus neutralization test.

Outcomes (Supplementary material, Appendix 2)

The primary outcome for phase IIa was safety, measured by the occurrence of serious adverse events over 28 days after vaccination; and for phase IIb, immunogenicity, evaluated by the successful immune response ($sVNT_{50}$ ≥250). It was assessed on days 0, 14 and 28 in phase IIa, and on days 0 and 28 in phase IIb.

Clinical laboratory tests performed on day 14 were compared to pre-vaccination values.

The secondary outcomes were reactogenicity and immunogenicity. Reactogenicity was assessed by the occurrence of solicited and protocol-defined local and systemic reactions, daily for seven days after vaccination, as well as unsolicited adverse events, daily for 28 days after vaccination. Vaccine immunogenicity was estimated after vaccination, and compared to baseline: The IgG anti-RBD ELISA and the SARS-CoV-2 neutralizing antibody ELISA were done on days 0, 14 and 28 in phase IIa, and on days 0 and 28 in phase IIb. Seroconversion rates and the inhibition ratio of RBD:hACE2 interaction were respectively estimated. The conventional live-virus neutralization test was performed on samples collected in both phases on days 0 and 28.

Statistical analysis

Calculation of the sample size for phase IIa was based on a serious adverse events rate lower than 5%. Two-sided 95% confidence intervals for one proportion were calculated, with a precision (target

width) of 0·250. In the phase IIb, the calculation of the sample size was based on a successful immune response of 50%; a lower limit of the confidence interval for the difference with respect to the control greater than 30%, and a randomisation ratio of 4:1. Two-sided 95% confidence intervals for the difference between two proportions with a target width of 0·200 were calculated. Finally, a 5% of sample size was added considering possible study withdrawals.

Safety and reactogenicity endpoints were described as frequencies (%). The following values were reported: mean, standard deviation (SD), median, interquartile range (IQR), and range for the demographic characteristics and adverse events. Median, 25^{th}-75^{th} percentile, geometric mean titres (GMT) and 95% confidence intervals (CI) for immunological endpoints. Seroconversion rates for IgG anti-RBD antibodies were calculated.

Spearman's rank correlation was used to assess relationships among techniques used to evaluate the immune response. The Student's t-Test or the Mann-Whitney U Test were used for before-after statistical comparison.

The assumption of normal distribution was checked by Kolmogorov-Smirnov test.

A stepwise logistic regression model was used to assess the influence of covariates on the successful immune response. A chi-square test was used to determine the association between two variables: the successful immune response induced by vaccination and independent variables (sex, race, age group, COVID-19 classification, hospital discharge time and inhibitory antibodies pre-vaccination), and between treatment and solicited adverse events.

A likelihood ratio —Bayes Factor— was used to carry out the risk-benefit analysis. Benefit was measured by the proportion of subjects with successful immune response induced by vaccination; risks were calculated by the serious and severe adverse events associated to vaccine (Supplementary material, Appendix 8).

Statistical analyses were done using SPSS version 25·0; EPIDAT version 4·1, Prism GraphPad version 6·0. A type I error of 0·05 was used.

Role of the funding source

Partial funding for this study was received from *Fondo de Ciencia e Innovación* (FONCI) of Cuba's Ministry of Science, Technology and Environment (Project-2020-20). Researchers of the Finlay Vaccine Institute —the Sponsor Centre— designed the study and participated in data analysis, interpretation, and writing the report. Researchers of the clinical sites, and other participating

institutions were responsible for the clinical trial execution and data collection. They contributed to data analysis and interpretation.

Results

From April 9, 2021, to April 17, 2021, 663 COVID-19 convalescent subjects were enrolled in the study; 213 participants were excluded for not meeting selection criteria and 450 volunteers were recruited. Twenty subjects aged 60-78 years were allocated into the open, non-controlled phase IIa, and received a single dose of FINLAY-FR-1A vaccine. Serious adverse events were not found, and successful immune response was found in 95% of subjects; therefore, inclusion of this age group in phase IIb was approved (Supplementary material, Appendix 6, Table 6-1).

Four-hundred and thirty subjects aged 19-78 years were randomised 4:1 to the experimental (N=344) and control groups (N=86) in phase IIb, and received a single dose of the vaccine or placebo respectively. There were three voluntary dropouts in the experimental group. Immunological results of eight subjects —three in the experimental group and five in the control group— could not be obtained; they could not be repeated, as not enough serum was available. All randomised subjects were included in the safety analysis (safety population), and the immunogenicity was evaluated in most subjects except those with study interruptions (per-protocol population) (Figure 1). The study ended on June 14, 2021.

Table 1 summarizes the demographic and baseline characteristics of the participants. There were no differences between the experimental and control groups. The mean time from hospital discharge to vaccination was 4·5 months (SD=3·3) in the experimental group, and 4·8 months (SD=3·9) in control group. Mild COVID-19 predominated in both groups.

The criteria for estimating the sample size were met. The sample size calculation in phase IIa was based on a serious adverse event rate of less than 0·05, and no serious adverse events were reported. The sample size calculation in phase IIb was based on a successful immune response of 50%; it was found in 81% of participants.

Local pain was the most frequent (29%) vaccine-associated adverse event, followed by swelling (4%). The main solicited systemic reactions were general malaise (7%) and headache (4%) (Table 2). The frequency of local and systemic reactions was higher during the first 24 h after vaccination; they generally disappeared within the first three days (Supplementary material, Appendix 4, Table 4-1, Table 4-2).

A significant association was detected between treatment and the occurrence of solicited adverse events ($p=0{\cdot}04$), where pain at the injection site was highly predominant ($p<0{\cdot}01$). No association was demonstrated between treatment (vaccine or placebo) and the other adverse events.

Serious vaccine-associated adverse events were not found. The intensity of the solicited adverse events was generally mild; only one subject (0·3%) reported a severe adverse event (headache), but recovered within the first hour after vaccination (Table 2). Five participants (1%) had moderate adverse events: local pain at the vaccination site (3), general malaise (1) and headache (1). Unsolicited adverse events were predominantly mild and resolved spontaneously during the follow-up period (Supplementary material, Appendix 4, Table 4-1, Table 4-2). Abnormal laboratory parameters related to vaccination were not found (Supplementary material, Appendix 5, Table 5-1).

A significant increase in RBD antibodies was detected after vaccination (median: 301·0 U/mL). Median value was six-fold higher than that of CCSP, 31-fold higher than the pre-vaccination level, and 46-fold higher than the control group ($p<0{\cdot}0001$). Seroconversion was 84% (Table 3), (Supplementary material, Appendix 6, Figure 6-1).

We measured the inhibition ratio of RBD:hACE2 interaction at a serum dilution of 1/100. On day 28 after vaccination, the levels of inhibitory antibodies were significantly higher than their pre-vaccination titres. The median of inhibitory antibody titres (94%) was three times greater than that of the CCSP and seven times greater than that of the control group ($p<0{\cdot}0001$) (Table 3) (Supplementary material, Appendix 6, Figure 6-2).

High levels of $sVNT_{50}$ were detected on day 28 post-vaccination; significantly higher to pre-vaccination titres, and to values from the control group and CCSP. The GMT of $sVNT_{50}$ on day 28 represents a 21-fold increase over the CCSP value, a 51-fold increase over the pre-vaccination value and a 45-fold increase over the control group ($p<0{\cdot}0001$) (Figure 2). The $sVNT_{50}\geq250$ was used to define successful immune response. It was found in most subjects (81%) immunized with FINLAY-FR-1A, versus only 5% in the control group ($p<0{\cdot}0001$) and 13% in the CCSP (Table 3). Most non-responders had a history of asymptomatic COVID-19 (61%), 39% had a history of mild disease.

We found an association between successful immune response and disease classification, as well as with time elapsed after hospital discharge. A higher number of vaccinated subjects with a successful immune response was found in moderate COVID-19 cases and in those with more than four months after hospital discharge ($p<0{\cdot}0001$). No association was found with sex, race, age and RBD:hACE2

inhibition rate before vaccination (p>0·05) (Supplementary material, Appendix 6, Table 6-2, Figure 6-3, Figure 6-4).

The conventional live-virus neutralization test was evaluated in 57 subjects: all subjects of phase IIa and 37 subjects of phase IIb. The GMT was 400·3, this represents a nine-fold increase over the CCSP (cVNT=46·4) and it was 26-fold higher than pre-vaccination titres (p<0·0001) (Table 3), (Supplementary material, Appendix 6, Figure 6-5). The vaccine induced neutralizing antibodies against the Alpha, Beta and Delta variants of the virus (Figure 3) (Supplementary material, Appendix 6, Table 6-3).

There was a good correlation of cVNT with other variables (coefficients greater than 0·7), except with RBD:hACE2 inhibition at a dilution of 1/100. The $sVNT_{50}$ and cVNT achieved the strongest correlation coefficient: 0·889; the correlation was 0·826 for cVNT and anti-RBD IgG concentration. Also, a strong correlation was found between $sVNT_{50}$ and anti-RBD IgG concentration (0·934), (Supplementary material, Appendix 7, Table 7-1).

The risk-benefit analysis showed strong evidence in favour of benefit. The odds were greater than 200, indicating that the probability of benefit is greater than the probability of risk (Supplementary material, Appendix 8, Figure 8-1, Figure 8-2).

Discussion

COVID-19 vaccines have been designed using several platforms: mRNA vaccines and viral vector vaccines are very immunogenic; however, there is concern regarding their reactogenicity.[13,22,23] The inactivated SARS-CoV-2 vaccines are less immunogenic, and concerns on their reactogenicity has been also reported.[13,24] Vaccines based on recombinant spike protein vaccines are also less immunogenic but provoke fewer adverse reactions.[13,15,25]

FINLAY-FR-1A (SOBERANA Plus) is based on recombinant d-RBD on aluminium hydroxide gel. It has been used as the third dose of a heterologous schedule in naïve subjects, after two first doses of FINLAY-FR-2 (SOBERANA 02); vaccine based on monomeric RBD units conjugated to tetanus toxoid as carrier protein.[26] After successful clinical trials, the National Regulatory Agency issued an emergency use authorisation this vaccination schedule in adults and children ≥2 years old. FINLAY-FR-1A (SOBERANA Plus) has also been studied as the third dose of a heterologous schedule in conjunction with the FINLAY-FR-1 (SOBERANA 01) vaccine, which is based on d-RBD adjuvanted with outer membrane vesicles of *Neisseria meningitidis* group B,[14] (a vaccination

schedule now under consideration by regulatory authorities). FINLAY-FR-1A (SOBERANA Plus) has also been used as a booster dose after prime-vaccination, and it has been studied for the protection of COVID-19 convalescent subjects against emerging SARS-CoV-2 variants.[15]

A key concern is the safety and reactogenicity of vaccines used in COVID-19 convalescents. A single dose of mRNA vaccines in SARS-CoV-2 seropositive individuals elicit a very rapid immune response, but there is an increase in adverse events. One study reported that 73% of US healthcare workers previously infected with SARS-CoV-2 had at least one adverse event.[27] In another study, adverse events were 89% more frequent in vaccinees with pre-existing immunity than in naïve subjects.[28,29] this may be due to a hypersensitivity reaction mediated by deposition of antigen-antibody immune complexes in tissues, which trigger an inflammatory reaction involving complement and leukocytes.

Here, only 32% of immunized individuals reported vaccine-associated adverse events, predominating local and mild events. Serious vaccine-associated adverse events were not detected.

This evaluation was carried out in a fragile population, persons who recently suffered from COVID-19, some with chronic disease, instead of in healthy naïve volunteers—as is usual in clinical trials. The low rate of adverse events and the absence of serious events confirmed its safety. We found fewer vaccine-associated adverse events than those reported in other studies.[22-24,30,31]

A 31-fold increase in anti-RBD IgG was detected over the pre-vaccination level. A similar finding was reported in other studies, proving stimulation of a secondary antibody response.[23,27,28,32-34] Seroconversion was 84%, slightly higher than that found on phase I (80%).[15]

Functional antibodies blocking RBD:hACE2 interaction were assessed in an *in-vitro* surrogate assay of the conventional live-virus neutralization test. The median inhibition value was 94%, the same we obtained in the phase I clinical trial performed in COVID-19 convalescents.[15]

The successful immune response was defined as the half-maximal surrogate virus neutralization titres ($sVNT_{50}$)≥250, assessed 28 days post-vaccination. This assay showed the best correlation with the live-virus neutralization test in the phase I clinical trial,[15] and here (0·889). The correlation between both tests has been verified, suggesting that this *in-vitro* test could replace the complex live-virus neutralization test.

The GMT of $sVNT_{50}$ on day 28 was notably higher than the pre-vaccination value, the control group and CCSP values, demonstrating the strong secondary immune response induced by FINLAY-FR-

1A vaccine. Most participants reached inhibitory antibody titres; 81% achieved a successful immune response.

The conventional live-virus virus neutralization test is considered the gold standard to evaluate neutralizing antibodies against SARS-CoV-2; a 26-fold increase over baseline titres evidences the efficacy of this vaccine in producing protective functional antibodies. Most individuals (82%) achieved cVNT>160, a value considered indicative of protection, similar to that of the phase I clinical trial,[15] and higher than the reported in other clinical trials.[24,30,31]

Live-virus neutralization test against the D614G variant was performed on a subset of 57 subjects. This variant was selected because it was the main circulating variant in the first two waves when participants in this study were infected.[14,15] As expected, most subjects had neutralizing antibodies before vaccination, which significantly increased post-vaccination, demonstrating stimulation of memory B cells.

A subsample of 10 subjects was further studied at the Hospital "Amedeo di Savoia" in Italy, with the inclusion of Alpha, Beta and Delta VOCs. The Omicron variant was not evaluated because it had not yet emerged at the time of the test.

The Alpha variant, initially reported in United Kingdom, has been associated with severe disease and mortality. The Beta variant, first documented in South Africa, has been associated with increases in hospitalizations and deaths, due to its ability to evade the vaccine-induced antibody response. In Cuba, this variant predominated during the first months of 2021. Delta emerged in India and is characterized by spread more easily. It is currently the predominant variant in Cuba and worldwide, along with the rapidly spreading Omicron variant.[1,7-10,26]

The immunological protection provided by COVID-19 vaccines or natural infection is being intensively studied.[7-10] While some studies reported natural protective immunity induced by SARS-CoV-2, reinfections have been reported in recovered subjects,[5,7-11,32,35] which seems increasing with the emergence of new VOCs.

As expected, low levels of neutralizing antibodies against Alpha, Beta and Delta VOCs were found before vaccination, especially the latter two, which increased considerably post-vaccination. Neutralizing antibodies against conserved epitopes could explain the large protective immune response against mutated SARS-CoV-2 variants induced by a single dose of FINLAY-FR-1A.

More convalescents achieved successful immune response when vaccinated beyond four months after hospital discharge with a negative PCR test, which could be related to lower levels of RBD

inhibitory antibodies that would prevent clearance of the vaccine antigen. However, there is not statistically evidence of association between RBD:hACE2 inhibitory antibodies detected before vaccination and a successful immune response (Supplementary material, Appendix 6, Table 6-2).

Ninety-five percent of phase IIa volunteers achieved a successful immune response after vaccination, compared to 81% when considering both trial phases together; however, this difference is not statistically representative (p=0·60), and no differences were found between the two age subgroups in the full trial (Supplementary material, Appendix 6, Table 6-2). There is some imbalance concerning the number of participants vaccinated >4 months after hospital discharge: 40% in phase IIa and 23% considering the full trial. Though the difference is not significant (p=0·07), it may be influencing the results and should be re-evaluated in upcoming clinical trials.

Symptomatic COVID-19 has been related to a stronger immune response compared to asymptomatic individuals,[4,5,32,36] and to a higher number of long-term memory B cells; this could explain the association between COVID-19 severity and $sVNT_{50}$ ≥250.

Most non-responders were asymptomatic or had a history of very mild COVID-19. Natural immunity probably controlled their disease, with low involvement of the B cell-mediated response and an insufficient generation of memory B cells. However, we cannot rule out effector T-cell activation in these subjects, as demonstrated in the phase I study of FINLAY-FR-1A in COVID-19 convalescents.[15]

This study confirms —now in convalescents— the immunogenicity of the FINLAY-FR-1A vaccine. B-cells were successfully stimulated 4·5 months on average after hospital discharge, with high levels of neutralizing antibodies, demonstrating that natural infection leads to the production of long-term memory B cells, and that a single dose induces a strong secondary immune response. Our results are in accordance with those of our phase I trial in convalescents,[15] as well as with another study, reporting that one year after infection, mRNA vaccines increase the immune response against SARS-CoV-2.[37]

The inclusion of a prime-vaccinated group in the study design would have been interesting for comparing the booster effect in this population with the response achieved in COVID-19 convalescents. Additional studies deserve the finding of higher neutralizing antibody titres in the 60-80 years age subgroup; due to the natural age-related decline of the immune response, this result should be further investigated.

Including COVID-19 convalescents with a history of severe disease should be also considered in further trials to evaluate potential association of the induced immune response with clinical severity of SARS-CoV-2 infection. The inclusion of younger age groups should be also considered in the design of upcoming clinical trials, as well as the evaluation of the Omicron variant and future emerging VOCs.

Although there is evidence of memory B-cell stimulation, based on a rapid induction of specific antibodies, we did not examine memory B- and T-cells and specific effector T-cells that should be studied by in vitro techniques

The efficacy and duration of the immune response elicited after viral infection is still under study. In our view, vaccination of previously infected individuals is necessary to protect them against new circulating variants. FINLAY-FR-1A (SOBERANA Plus) could be an important tool against COVID-19, especially to strengthen pre-existing immunity secondary to infection or vaccination.

Contributors' Roles

ROA and ACM are joint first authors. ROA, ACM, CMA, YCR, and VVB contributed equally. ACM was the principal investigator and ROA was the co-principal investigator of this trial. ROA, CMA, CVS, YVB, DGR, GWC, and VVB conceived the study, designed the trial, the study protocol, and were involved in data analysis and interpretation. YCR, ROA and PPGC supervised and monitored the trial. ACM, CMA, MAGG, YJB, YTM, LRV and LRP RPG were responsible for the site work including the recruitment and data collection. They contributed to data analysis and interpretation. LRN, BSR, THG, IOV, MDH, MRA, ENR, JEP, DOL, IVA, ADF, APD, FC, AC and VG carried out immunological experiments and the analysis of results. ACM, CVS, RGM and ROA had access to the raw data. CVS and ROA verified the data. CVS and RGM were involved in data curation and statistical analysis of data. ROA and VVB wrote the manuscript, and all authors provided paper feedback. ROA has final responsibility for publication.

Declaration of Interests

The Finlay Vaccine Institute and the Centre of Molecular Immunology manufacture the vaccine and have filed patent applications related to the vaccine's use in individuals with pre-existing SARS-CoV-2 immunity. VVB, YVB, DGR, ROA, YCR, BSR, MDH, IOV, CMA, ACM and MRA are authors of these patent applications. ROA, YCR, LRN, RGM, YVB, DGR, VVB, BSR, THG, IOV and MDH are researchers of the Centres that manufacture the vaccine. Partial funding for this study

was received from *Fondo de Ciencia e Innovación* (FONCI) of Cuba's Ministry of Science, Technology and Environment (Project-2020-20). The other authors declare no competing interests. No authors received an honorarium for this paper.

Data sharing

Data about adverse events and immune response are shared in the Supplementary Material. Some information is also available at the Cuban Public Registry of Clinical Trials, included in WHO International Clinical Trials Registry Platform (Soberana Plus, https://rpcec.sld.cu/en/trials/RPCEC00000366-En). The individual immunological and safety data, as well as other supporting clinical documents, including study protocol, statistical analysis plan, and the informed consent form will be available after publication of this article. Proposals should be sent to: ochoa@finlay.edu.cu or: vicente.verez@finlay.edu.cu. These proposals must be reviewed and approved by the sponsor and the investigator. Finally, a data access agreement must be signed.

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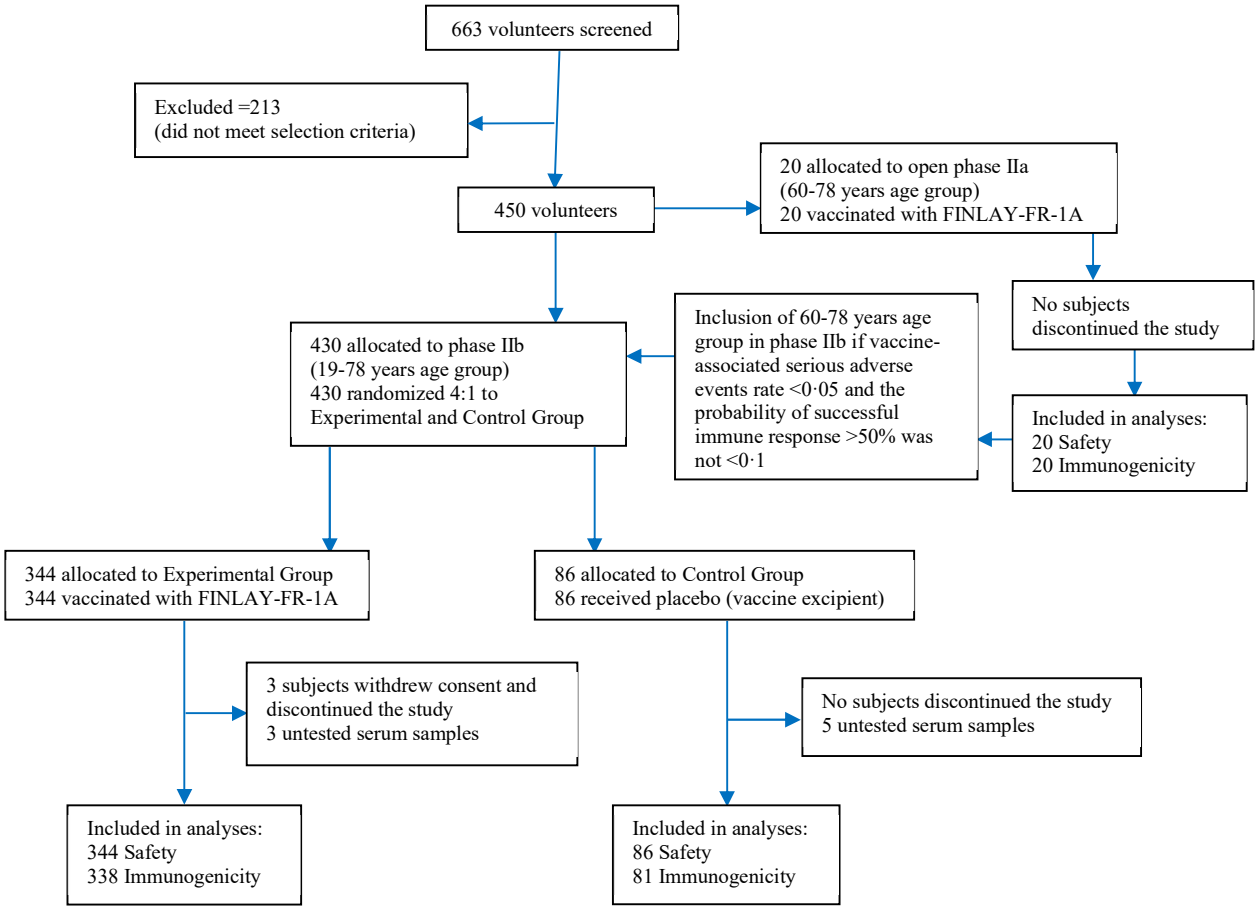


***Figure 1*: Trial profile.**

The study was sequentially performed in two stages: phase IIa: open, non-controlled; phase IIb: randomised, placebo-controlled, and double-blind. FINLAY-FR-1A (SOBERANA Plus) vaccine: 50 µg of dimeric-Receptor Binding Domain in aluminium hydroxide gel. Placebo: vaccine excipient. Successful immune response: half-maximal surrogate virus neutralization titres ≥250.

	Experimental Group	Control Group
N	364	86
Sex		
Female	204 (56%)	47 (55%)
Male	160 (44%)	39 (45%)
Race		
White	224 (62%)	55 (64%)
Black	54 (15%)	11 (13%)
Mixed race	85 (23%)	20 (23%)
Yellow	1 (0·3%)	0 (0%)
Age (years)		
Mean (SD)	46·0 ± 14·3	45·0 ± 14·3
Median (IQR)	49·0 ± 24·0	45·0 ± 23·0
Range	19-78	21-78
19-59 age group	305 (84%)	77 (90%)
60-78 age group	59 (16%)	9 (11%)
Weight (kg)		
Mean (SD)	74·5 ± 15·0	73·7 ± 14·6
Median (IQR)	74·0 ± 21·0	73·0 ± 21·1
Range	44·0-130·0	44·0-105·0
Height (cm)		
Mean (SD)	166·0 ± 9·0	165·6 ± 10·0
Median (IQR)	165·0 ± 12·0	166·0 ± 1·3
Range	147-198	145-190
BMI (kg/m^2)		
Mean (SD)	26·9 ± 4·3	26·8 ± 4·2
Median (IQR)	27·0 ± 6·5	27·0 ± 6·4
Range	18·4-35·3	18·3-34·7
HD (months)		
Mean (SD)	4·5 ± 3·3	4·8 ±3·9
Median (IQR)	3·1 ±1·3	3·0 ±1·4
Range	1·8-15·9	2·0-15·5
COVID-19 classification		
Asymptomatic	85 (23%)	25 (29%)
Mild	245 (67%)	38 (44%)
Moderate	34 (9%)	23 (27%)

Experimental Group: vaccinated with FINLAY-FR-1A (SOBERANA Plus). Control Group: injected with the vaccine excipient. Data are n (%). Mean (SD): Mean ± Standard Deviation. Median (IQR): Median ± Interquartile Range. BMI: body mass index. HD: months from hospital discharge with negative-PCR test to vaccination.

***Table* 1: Baseline characteristics of the COVID-19 convalescents included in the study**

	Experimental Group	Control Group
N	364	86
Subjects with some TAAE	117 (32%)	18 (21%)
Subjects with some Serious TAAE	0 (0%)	0 (0%)
Subjects with some Severe TAAE	1 (0·3%)*	0 (0%)
Solicited local TAAE		
Site pain	105 (29%)	13 (15%)
Swelling	16 (4%)	4 (5%)
Local heat	14 (4%)	0 (0%)
Induration	11 (3%)	1 (1%)
Redness	8 (2%)	0 (0%)
Solicited systemic TAAE		
General malaise	24 (7%)	7 (8%)
Headache	15 (4%)	1 (1%)
Somnolence	8 (2%)	1 (1%)
Fever	2 (1%)	1 (1%)
Limitation of activity	0 (0%)	1 (1%)
Unsolicited systemic TAAE		
Dizziness	1 (0·3%)	0 (0%)
Diarrhoea	1 (0·3%)	0 (0%)
Asthenia	0 (0%)	1 (1%)
Nasal discharge	1 (0·3%)	1 (1%)
Fatigue	1 (0·3%)	0 (0%)
Cough	1 (0·3%)	0 (0%)
dyspnoea	1 (0·3%)	0 (0%)
Bilateral conjunctival injection	1 (0·3%)	0 (0%)
Chills	1 (0·3%)	0 (0%)
Number of TAAE per subject		
Average (SD)	0·6 ± 1·0	0·4 ± 0·8
Median (IQR)	0 ± 1	0 ± 0
Range	0-5	0-4

Experimental Group: vaccinated with FINLAY-FR-1A (SOBERANA Plus). Control Group: injected with the vaccine excipient. TAAE: Treatment-Associated Adverse Event. Data are n (%) unless otherwise specified. Average (SD): Average ± Standard Deviation. Median (IQR): Median ± Interquartile Range.

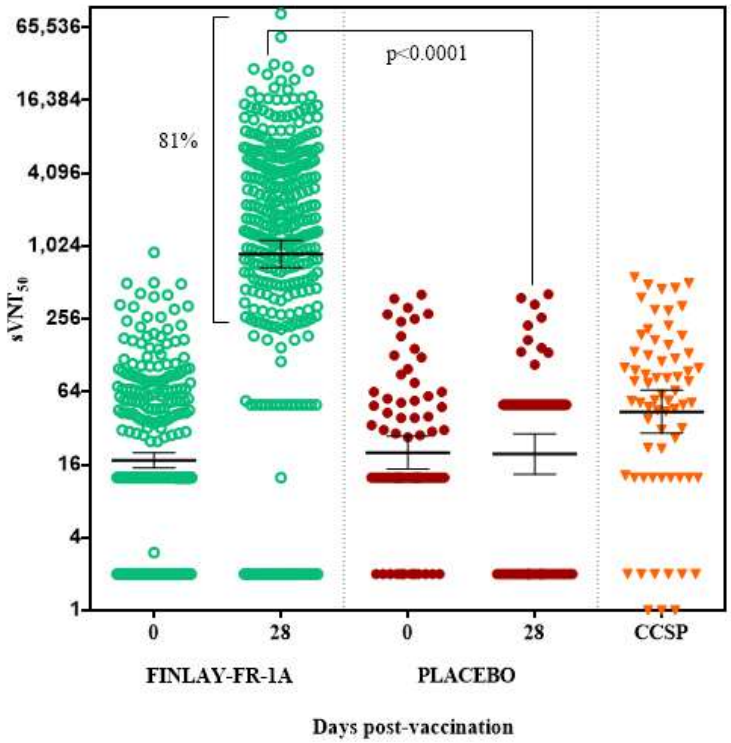
*Headache that impedes activities.

***Table* 2: Frequency of treatment-associated adverse events**

	Experimental Group		Control Group		CCSP
	T0	T28	T0	T28	
Anti-RBD IgG U/mL					
Median	9·7	301·0	10·2	6·6	50·8
25-75 percentile	3·0; 28·8	103·0; 819·2	2·5; 25·7	1·9; 17·1	23·8; 94·0
Anti-RBD IgG Seroconversion					
n (%)	N.A.	302 (84)	N.A.	0 (0)	N.A.
95% CI	N.A.	80; 88	N.A.	0; 1	N.A.
RBD:hACE2 INH %					
Median	11	94	12	13	32
25-75 percentile	4; 27	89; 95	5; 26	6; 22	17; 62
$sVNT_{50}$					
GMT	17·4	884·0	20·1	19·6	41·8
95% CI	15·0; 20·1	682·1; 1145·7	14·8; 27·4	13·3; 28·8	27·7; 63·2
$sVNT_{50} \geq 250$					
n (%)	13 (4)	289 (81)	6 (7)	4 (5)	9 (13)
95% CI	2; 6	76; 85	3; 15	1; 12	6; 24
cVNT					
GMT	15·4	400·3	N.A.	N.A.	46·4
95% CI	10·3; 23·2	272·4; 588·1	N.A.	N.A.	31·5; 68·4

Experimental Group: vaccinated with FINLAY-FR-1A (SOBERANA Plus). Control Group: injected with the vaccine excipient. T0: pre-vaccination. T28: 28 days post-vaccination. U/mL: anti-RBD IgG concentration expressed in units/mL. Anti-RBD IgG Seroconversion: ≥4-fold increase in antibody titres over pre-immunization titres. RBD:hACE2 INH%: RBD:hACE2 inhibition % at a dilution 1/100. $sVNT_{50}$: serum dilution inhibiting 50% of RBD:hACE2 interaction. $sVNT_{50} \geq 250$ was defined as "successful immune response". cVNT: conventional live-virus neutralization titre. GMT: Geometric Mean Titre. 95% CI: 95% Confidence Interval. N.A.: not applicable. CCSP: Cuban convalescent serum panel.

***Table* 3: Humoral immune response induced by a single dose of FINLAY-FR-1A vaccine**



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640 ***Figure 2*: Half-maximal surrogate virus neutralization titre ($sVNT_{50}$).**

641 $sVNT_{50}$ is the reciprocal serum dilution giving 50% inhibition of RBD:hACE2 interaction, measured by

642 competitive ELISA at days 0 (pre-vaccination) and 28. CCSP: Cuban Convalescent Serum Panel.

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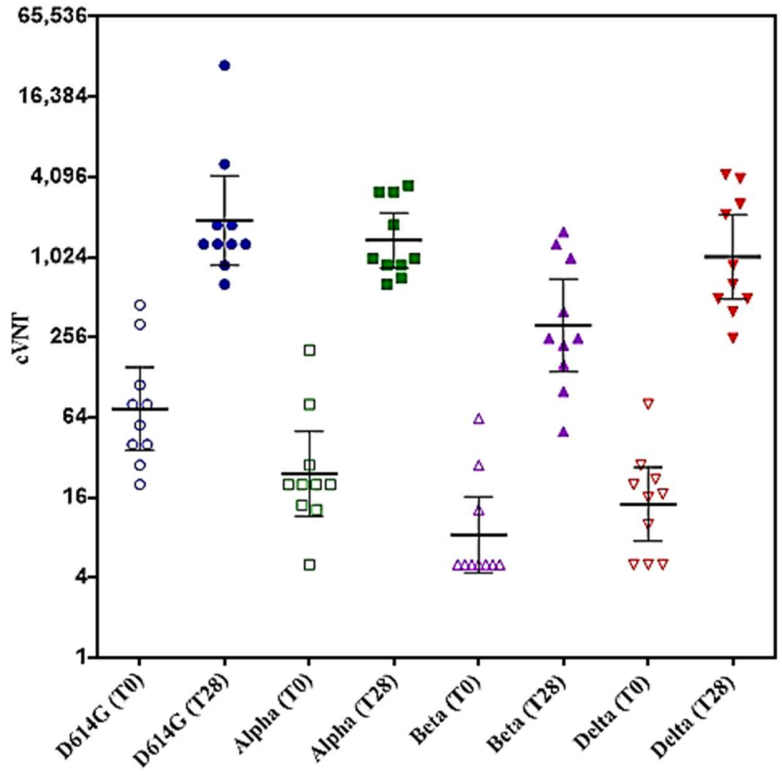

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646 ***Figure 3*: Titres of neutralizing antibodies (cVNT) against four SARS-CoV-2 variants of concern at**
647 **days 0 (pre-vaccination) and 28 (post-vaccination).**

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