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Research paper

A single dose of SARS-CoV-2 FINLAY-FR-1A vaccine enhances neutralization response in COVID-19 convalescents, with a very good safety profile: An open-label phase 1 clinical trial

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A R T I C L E   I N F O

A B S T R A C T

Background: As a first step towards a vaccine protecting COVID-19 convalescents from reinfection, we evaluated FINLAY-FR-1A vaccine in a clinical trial.

Methods: Thirty COVID-19 convalescents aged 22-57 years were studied: convalescents of mild COVID-19, asymptomatic convalescents, both with PCR-positive at the moment of diagnosis; and individuals with subclinical infection detected by viral-specific IgG. They received a single intramuscular injection of the FINLAY-FR-1A vaccine (50 μg of the recombinant dimeric receptor binding domain). The primary outcomes were safety and reactogenicity, assessed over 28 days after vaccination. The secondary outcome was vaccine immunogenicity. Humoral response at baseline and following vaccination was evaluated by ELISA and live-virus neutralization test. The effector T cellular response was also assessed. Cuban Public Registry of Clinical Trials, WHO-ICTRP: https://rpcec.sld.cu/en/trials/RPCEC00000349-En.

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Findings: No serious adverse events were reported. Minor adverse events were found, the most common, local pain: 3 (10%) and redness: 2 (6.7%). The vaccine elicited a >21 fold increase in IgG anti-RBD antibodies 28 days after vaccination. The median of inhibitory antibody titres (94.0%) was three times greater than that of the COVID-19 convalescent panel. Virus neutralization titres higher than 1:160 were found in 24 (80%) participants. There was also an increase in RBD-specific T cells producing IFN-γ and TNF-α.

Interpretation: A single dose of the FINLAY-FR-1A vaccine against SARS-CoV-2 was an efficient booster of pre-existing natural immunity, with excellent safety profile.

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Research in context

Evidence before this study

Immunity against SARS-CoV-2 depends on the level of neutralizing antibodies. Asymptomatic individuals and persons recovered from mild disease may be reinfected, particularly those with low-neutralizing antibody titres. As far as we know, SARS-CoV-2 vaccines are not being evaluated in clinical trials for preventing reinfection in COVID-19 convalescents. There is strong evidence that COVID-19 induces long-term memory B cells that can respond to RBD vaccines.

Added value of this study

This is the first published clinical study of an anti-SARS-CoV-2 vaccine in 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 7 days after a single vaccine dose, which proves booster effect over existing immunity. There was also an increase in RBD-specific T cells producing IFN-γ and TNF-α. B and T cells were successfully stimulated 8 months on average after hospital discharge or serological diagnosis, demonstrating that natural infection leads to the production of long-term memory cells that can respond quickly to a booster dose of FINLAY-FR-1A vaccine.

Implications of all the available evidence

A d-RBD vaccine can be used as a booster 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 this vaccine triggered a rapid induction of high cellular and humoral response, suggesting a protective immunity against COVID-19, which should be confirmed in large phase II clinical trials.

1. Introduction

By mid-August 2021, the number of COVID-19 cases reported worldwide is about 205 million and the number of persons recovered is approaching 175 million [1]. Disease severity goes from asymptomatic and mild to severe with fatal outcome, mainly in persons with impaired immunity and comorbidities in which an uncontrolled inflammatory response and cytokine storm are responsible for a torpid evolution [2-5].

COVID-19 convalescents are not included in vaccination programs and there is insufficient understanding of the efficiency and duration of protection conferred via natural immunity induced by SARS-CoV-2 infection. Depending on the level of neutralizing antibodies, evidence points to short or to long-term immunity [4-10].

Other studies provide evidence of reinfection [8, 9]. What are the pros and cons of vaccinating convalescents? Do they develop adverse events, not observed in the naïve population? Can they be protected against reinfection?

Neutralizing antibodies against SARS-CoV-2 are stimulated by the S1 subunit of the spike protein, mainly by its receptor binding domain (RBD), while other SARS-CoV-2 proteins can promote an immunopathogenic mechanism mediated by antibodies (Antibody Dependent Enhancement, ADE) [2, 3, 8, 10].

Vaccine candidates based on RBD have been developed on different platforms, which have demonstrated safety and immunogenicity [9, 11, 12]. FINLAY-FR-1A vaccine (SOBERANA Plus), produced under Good Manufacturing Practice at The Finlay Vaccine Institute and The Centre of Molecular Immunology, in Havana, Cuba has completed preclinical and toxicological evaluations. The antigen is a dimer of the recombinant RBD with sequence 319-54, produced in genetically modified Chinese hamster ovary cells (CHO). RBD is dimerized through a Cys538–Cys538 interchain disulphide bridge.

The dimeric RBD (d-RBD) was developed as an alum-adsorbed vaccine —FINLAY-FR-1A— or combined with alum and outer membrane vesicles from *Neisseria meningitidis* group B as adjuvant. The FINLAY-FR-1A vaccine was evaluated in a Phase I Clinical Trial in naïve individuals. Two formulations were tested: 25 μg and 50 μg of d-RBD per dose in a 3-dose schedule. This study showed an excellent safety profile of both formulations, as well as higher immunogenicity of the 50 μg formulation (Cuban Public Registry of Clinical Trials: https://rpcec.sld.cu/en/trials/RPCEC00000338-En, included in WHO International Clinical Trials Registry Platform). This phase I clinical trial was successfully completed, and the National Regulatory Agency authorized the start of new trials.

We hypothesize that a single dose of this vaccine may be an effective booster for individuals with pre-existing immunity to SARS-CoV-2. Here we describe the safety and immune responses after application of a single dose of FINLAY-FR-1A vaccine to 30 individuals with documented pre-existing SARS-CoV-2 natural immunity.

2. Methods

2.1. **Study design and participants** *(see also Supplementary Material, Appendix 2)*

This phase I, adaptive, open, and monocentric clinical trial was carried out at the National Institute of Haematology and Immunology in Havana, Cuba. The clinical trial protocol is available at https://rpcec.sld.cu/en/trials/RPCEC00000349-En (hereinafter referred to as *the protocol*).

The adaptive design planned the following prospective adaptations:

- Stopping rule for unacceptable toxicity (if the probability of vaccine-associate serious adverse events rate were greater than 0·05).
- Early evaluation of immunogenicity (if the probability of immune response were greater than 0·90 a report would be submitted to the regulatory agency for advancing in the design for the next study).
- Inclusion of other evaluation criteria depending of the external accumulated data.

Thirty convalescent individuals of COVID-19 were recruited among COVID-19 convalescent individuals in Havana who fulfilled the selection criteria (see also Supplementary Material, Appendix 2). The time elapsed from hospital discharge or serological diagnosis to vaccination was computed. Participants were distributed into three groups: convalescents of mild COVID-19 (N=11), asymptomatic convalescents (N=10), both with positive PCR test at the moment of diagnosis and cleared at least two months before the initiation of the study (a safety requirements of the Cuban protocol for convalescent patients) [13], and individuals with subclinical infection detected by community-based research with SARS-CoV-2-specific IgG but who never were confirmed as PCR positive (N=9) [13]. COVID-19 convalescents of the first two groups had history of hospital admission in accordance with the Cuban Protocol [13]. The subjects of the third group were identified during seroepidemiological studies addressed to people without history of clinical manifestations of COVID-19. (See also Supplementary Material, Appendix 4, Table 4-2).

All participants underwent a screening visit (full medical history, pregnancy rapid test in women of childbearing potential, SARS-CoV-2 PCR tests, blood tests —HIV; hepatitis B and C serology; full blood count; kidney and liver function tests, background of IgG anti-RBD antibodies, blocking antibodies of RBD:hACE2 interaction, virus neutralization test and cellular immunity—). Exclusion criteria were: for safety reasons, history of moderate or severe COVID-19 hospitalization due to COVID-19 during the last 2 months, and any severe disease or decompensated chronic disease, immunodeficiency, history of serious allergy, pregnancy, breastfeeding, immunological treatment during the last 30 days; SARS-CoV-2 PCR-positive, detection of antibodies blocking RBD:hACE2 interaction higher than 60% at a serum dilution 1/100. (See also Supplementary Material, Appendix 2). The study was registered at the Cuban Public Registry of Clinical Trials: https://rpcec.sld.cu/en/trials/RPCEC00000349-En, included in WHO International Clinical Registry Trials Platform.

2.2. Ethical considerations

The Cuban Ministry of Public Health (MINSAP) established a medical care program for COVID-19 convalescent patients [13]. The National Institute of Haematology and Immunology (NIHI) —clinical site of the trial— and the trial's clinical research team are included in this medical care program. MINSAP, the Independent Ethics Committee for Studies on Human Subjects, at NIHI 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: 30/12/2020, Reference number: 542/05-017-20B). It was conducted according to the Declaration of Helsinki and Good Clinical Practice.

An Independent Data Monitoring Committee (four members 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. After day 28, the final analysis of safety, reactogenicity, and immunogenicity were done by the statistician responsible of the design and statistical analysis. All subjects were studied during the interim analysis on day 14 and during the final analysis —whole trial— after day 28.

During recruitment, investigators provided potential participants with extensive relevant information, both oral and written. All questions and doubts were clarified. The decision to participate in the study was completely voluntary. 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.

2.3. 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, purified and characterized as usual. RBD is dimerized through a Cys538–Cys538 interchain disulphide bridge. FINLAY-FR-1A vaccine, composition per dose (0·5 mL): d-RBD 50 μg, NaCl 4·250 mg, Na_2HPO4 0·03 mg, NaH_2PO4 0·02 mg, thimersal 0·05 mg, injection water, aluminium hydroxide gel 1·25 mg, pH 6·0–7·2. The vaccine was manufactured according to Good Manufacturing Practice by the Finlay Vaccine Institute in Havana, Cuba.

2.4. Procedures

Blood samples were collected on days 0 (before vaccination), 7, 14 and 28. Volunteers were closely observed for 3 hours post-vaccination. After vaccination, active surveillance by health care professionals was carried out on days 1 (vaccination), 2, 3, 7, 14 and 28. Participants were instructed to complete a diary record of solicited local and systemic adverse reactions during the 28 days follow-up period.

Expected 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 $\geq$38°C) were recorded for 7 days. All other events, —in particular, possible serious adverse events— were recorded throughout the 28 days follow-up period. The severity 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. Severity of unsolicited adverse events were graded as: mild (transient or mild discomfort, no interference with activity), moderate (mild to moderate limitation in activity), severe (marked limitation in activity). 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 [14].

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

a) *in-house quantitative IgG ELISA* to detect antibodies against d-RBD, using d-RBD as coating antigen. The assay uses an in-house standard characterized serum, which was arbitrarily assigned 200 AU/mL (based on a half-maximal inhibitory titre of 200 and conventional virus neutralization titre of 160). The standard curve was constructed by performing six two-fold serial dilutions (1:100 to 1:1600). An anti-human-γ:peroxidase conjugate was used; the reference curve was constructed using four-parameter log-logistic function of the Centers for Disease Control and Prevention Program [15]. (See also Supplementary Material, Appendix 9).

b) *Molecular virus neutralization test*, based on antibody-mediated blockage of RBD:hACE2 interaction. This test is an in-vitro surrogate of the live-virus neutralization test [16]. 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 murine conjugated to alkaline phosphatase. This inhibition ELISA mimics the virus-host interaction at the molecular level [16]. The inhibition ratio of RBD:hACE2 interaction at a serum dilution of 1/100 and the half-maximal molecular virus neutralization titre (mVNT$_{50}$) were calculated; mVNT$_{50}$ is the serum dilution inhibiting 50% of RBD:hACE2 interaction.

c) *Conventional virus neutralization test*. This live-virus neutralization 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 cytophatic effect on Vero E6 cells. The conventional virus neutralization titres (cVNT) were calculated [17].

Measurement of cellular response. After vaccination, RBD-specific T cells producing IFN-γ and TNF-α were quantified by multiparametric intracellular flow cytometry [18]. Briefly, peripheral blood mononuclear cell (PBMC) were isolated, cultured in the presence of full-length recombinant RBD [18], Brefeldin A solution was added, cells were collected and stained first with the live/dead near-IR fluorescent dye (Invitrogen), and then, with the extracellular markers anti-CD3 PE/cy7 (SK7) and anti-CD4 PE/cy5 (RPA-T4). Cells were fixed, permeabilized, and stained with anti-human IFN-γ PE (4S.B3) and TNF-α FITC (mAb11). Lymphocytes were acquired in a Gallios cytometer and data were analysed using the Kaluza 1·2 version software. Cytokine-producing T lymphocytes were gated on PBMC [18].

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

2.5. Outcomes *(See also Supplementary Material, Appendix 1)*

The two co-primary outcomes, safety, and reactogenicity, were assessed over 28 days after vaccination. Safety was measured by the occurrence of serious adverse events. Preliminary results were assessed by the occurrence of expected and protocol-defined local and systemic reactions, as well as unsolicited adverse events, on days 7 and 14 after vaccination; the final evaluation was performed on day 28 after vaccination. Laboratory tests on day 28 were compared to pre-vaccination values.

The secondary outcome, vaccine immunogenicity, was estimated on days 7, 14, and 28, and compared to baseline. The IgG anti-RBD ELISA and the molecular virus neutralization test were done on days 0, 7, 14 and 28; the conventional virus neutralization test on days 0 and 14, and the evaluation of cellular immunity on days 0 and 28.

Cellular immunity was evaluated in subjects of the first two groups with clear history of COVID-19, (who were admitted to hospital and confirmed by PCR). Humoral immunity was studied in all participants, including subjects without history of clinical symptoms of COVID-19 and with negative-PCR tests, not hospitalized but with history of positive IgG COVID-19 tests.

2.6. Statistical analysis

Calculation of the sample size 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 0·194.

Safety and reactogenicity endpoints are described as frequencies (%). The following values are reported: mean, standard deviation (SD), median, interquartile range, and range, for the demographic characteristics and adverse events. Median, for immunological endpoints; geometric mean (GMT) and 95% confidence intervals (CI), for mVNT$_{50}$ and cVNT. Seroconversion rates for IgG antibodies anti-RBD ($\geq$4-fold increase in antibody concentration over pre-immunization levels) were calculated for each subject.

Spearmańs rank correlation was used to assess relationships among techniques used to evaluate the immune response. ROC curve was used to choose the most appropriate cut-off for humoral tests regarding the cVNT, and to determine the connection between sensitivity and specificity for every cut-off. The Studentś t-Test or the Wilcoxon Signed-Rank Test were used for before-after statistical comparison. Statistical analyses were done using SPSS version 25·0; STATISTICA version 12·0; R version 3·2·4; EPIDAT version 4·1, Prism GraphPad version 6·0 and WinBugs version 1·4. An alpha signification level of 0·05 was used.

An Independent Data and Safety Monitoring Board provided safety supervision.

3. Results

Table 1 summarizes the participants' demographic characteristics. The mean time from hospital discharge or serological diagnosis to vaccination was 8 months (SD=1·2), the median value was 8·2 months. (See also Supplementary Material, Appendix 4, Table 4-2).

The sample size calculation was based on a serious adverse event rate of less than 0·05. No serious adverse events were reported. The probability of vaccine-related serious adverse events was estimated as 0·032 and the probability of reaching an unacceptable toxicity (greater than 0·05) was 0·20.

Local pain was the most frequent (10%) minor adverse event, followed by redness (6·7%) (Table 2). Both were the only expected local adverse events with consistent causal association to vaccination. The expected systemic reactions were limited to general malaise with causality association and mild fever, with inconsistent causal association to vaccination. Only six subjects (20%) reported adverse events (one reported two local reactions: local pain and redness) (Table 2). Abnormal laboratory parameters related to vaccination were not found. (See also Supplementary Material, Appendix 5, Tables 5-1 to 5-4, Appendix 6, Table 6-1).

The frequency of local and systemic reactions was higher during the first 24 h after vaccination; they generally disappeared within the first 3 days. The intensity of the solicited adverse events was generally mild; only two participants reported moderate local pain at the vaccination site. Unsolicited adverse events were predominantly mild and moderate and resolved spontaneously during the follow-up period. (See also Supplementary Material, Appendix 5, Tables 5-1 to 5-4). The main unsolicited adverse event was high blood pressure; in only one case (3·3%) consistent with causal association to vaccination. This subject was the only severe case in this clinical trial, but recovered within the first hour after vaccination. Volunteers with a history of high blood pressure were admitted to the study if blood pressure remained controlled during recruitment.

A significant increase in RBD antibodies was detected on day 7 (median: 146·6 AU/mL). IgG level increased on days 14 and 28, with medians of 330·4 and 722·2 AU/mL respectively (Table 3 and Figure 1). The vaccine elicited a very high increase in antibody response on day 28. Median values were 14-fold higher than that of CCSP and 21-fold higher than the pre-vaccination level. Seroconversion was 50% at 7 days. It was 66·67% and 80% at 14, and 28 days respectively. (See also Supplementary Material, Appendix 7, Tables 7-1 to 7-3).

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

	Subjects recovered from mild COVID-19	History of PCR-positive asymptomatic COVID-19	Past subclinical infection detected by viral-specific IgG	Total
N	11	10	9	30
Sex				
Female	7 (63·6%)	3 (30·0%)	5 (55·6%)	15 (50·0%)
Male	4 (36·4%)	7 (70·0%)	4 (44·4%)	15 (50·0%)
Skin color				
White	8 (72·7%)	5 (50·0%)	3 (33·3%)	16 (53·3%)
Black	1 (9·1%)	2 (20·0%)	2 (22·2%)	5 (16·7%)
Mixed race	2 (18·2%)	3 (30·0%)	4 (44·4%)	9 (30·0%)
Age (years)				
Mean (SD)	46·9 ± 8·8	36·6 ± 10·4	39·7 ± 14·0	41·3 ± 11·6
Median (IQR)	48·0 ± 13·0	33·0 ± 14·0	40·0 ± 30·0	41·5 ± 21·0
Range	30-57	24-55	22-57	22-57
Weight (kg)				
Mean (SD)	76·8 ± 14·6	70·0 ± 12·0	70·1 ± 13·2	72·5 ± 13·3
Median (IQR)	75·0 ± 29·0	71·0 ± 17·0	71·0 ± 14·5	71·0 ± 12·1
Range	55-100	47-90	46-92	46-100
Height (cm)				
Mean (SD)	169·3 ± 12·5	167·9 ± 8·2	167·2 ± 10·7	168·2 ± 10·4
Median (IQR)	163·0 ± 23·0	169·0 ± 13·0	164·0 ± 19·0	166·0 ± 16·0
Range	153-188	152-177	154-186	152-188
BMI (kg/m²)				
Mean (SD)	26·5 ± 1·9	24·6 ± 2·8	24·8 ± 3·4	25·4 ± 2·8
Median (IQR)	26·0 ± 3·7	24·4 ± 4·4	26·4 ± 4·9	25·6 ± 4·2
Range	23·5-33·7	20·3-29·7	18·7-29·4	18·7-29·7
HD (months)				
Mean (SD)	8·4 ± 0·6	7·8 ± 1·7	7·9 ± 0·9	8·0 ± 1·2
Median (IQR)	8·5 ± 1·0	8·2 ± 1·7	8·2 ± 0·1	8·2 ± 0·8
Range	7·4-9·4	4·7-10·1	5·5-8·3	4·7-10·1

Data are n (%) unless otherwise specified. Mean (SD)=Mean ± Standard Deviation. Median (IQR)=Median ± Interquartile Range. BMI=Body mass index. HD=Months from hospital discharge or serological diagnosis to vaccination.

We measured the inhibition ratio of RBD:hACE2 interaction at a serum dilution of 1/100. Twenty-six subjects (86·6%) presented levels of inhibitory antibodies on day 7. Significantly higher than their pre-vaccination titres, and those from CCSP (Figure 2). On day 7, 23 (76·67%) individuals achieved an inhibition ratio >70% at a serum dilution of 1/100. On day 14, 26 participants (86·67%) had responded to vaccination; they attained a 94% inhibition ratio and the median of the inhibitory antibody titres was three times greater than that from CCSP (Table 3). (See also Supplementary Material, Appendix 7, Tables 7-5).

To evaluate the functionality of antibodies, the $mVNT_{50}$ value complements the information derived from the RBD:hACE2 inhibition ratio. High levels of $mVNT_{50}$ were detected on day 7 post-vaccination (significantly higher to pre-vaccination titres and to the convalescent serum panel) (Figure 3). The GMT of $mVNT_{50}$ on day 28 represents a 103-fold increase over the pre-vaccination value (2243·2/21·7) and a 38-fold increase over the CCSP value (2243·2/59·3). (See also Supplementary Material, Appendix 7, Tables 7-6).

The conventional virus neutralization titre (cVNT) obtained with live SARS-CoV-2 was evaluated on day 14. The GMT —calculated in all sample subjects— was 234·3, this represents a 5-fold increase over the value for CCSP (cVNT=46·4) (Table 3). After vaccination, virus neutralization titres higher than 1:160 were found in 24 (80%) participants. They were significantly higher than pre-vaccination titres, and CCSP titres (Figure 4). (See also Supplementary Material, Appendix 7, Tables 7-4).

The cellular immunity was explored in convalescent subjects with a history of PCR-positive COVID-19. Anti-CD3 and anti-CD4 monoclonal antibodies were used to identify lymphocyte subsets. The vaccine increased the frequency of RBD-specific TNF-α T cells on day 28. The expression of TNF-α from CD4+ and CD3+CD4- T cells was significantly higher than at baseline (p=0·00014; 95% CI:

0·43; 1·09 and p=0·00025; 95% CI: 1·19; 4·29, respectively). The frequency of RBD-specific IFN-γ CD3+CD4- T cells also increased, (p=0·030; 95% CI: 0·03; 1·09). These are evidence of a vaccine-triggered cellular immune response (Figure 5). (See also Supplementary Material, Appendix 7, Table 7-7)

Only four participants (all women) did not increase antibody level after vaccination (one convalescent from mild COVID-19, one asymptomatic; the other two, convalescents from subclinical infection (viral-specific IgG, PCR-negative). Before vaccination, they had a very low level of anti-RBD antibodies. The two women with history of mild COVID-19 and the one asymptomatic increased CD3+CD4- TNF-α T cells (probably CD8+ T cells) after vaccination. The last one also increased the CD3+CD4- IFN-γ T cells. (See also Supplementary Material, Appendix 7, Tables 7-8, 7-9).

For all immunological endpoints analysed, no differences were found among the three groups. Although the sample size is small, the 95% confidence intervals or 25-75 percentile ranges of each group overlap, suggesting similarity in the immune response.

There was a good correlation between cVNT and other variables (coefficients greater than 0·7), except with RBD:hACE2 inhibition at a dilution of 1/100. mVNT50 and cVNT achieved the strongest correlation coefficient: 0·946 (95% CI: 0·889; 0·974); the correlation was 0·936 (95% CI: 0·869; 0·969) for cVNT and anti-RBD IgG concentration, and 0·730 (95% CI: 0·502; 0·863) for cVNT and the seroconversion rate.

The best predictive result was for $mVNT_{50}$ (cut-off value: 919). The diagnostic efficiency of cVNT was 96·7% (95·8% sensitivity, 100% specificity, 100% positive predictive value, and 85·7% negative predictive value), followed by anti-RBD IgG concentration (cut-off value: 124·7 AU/mL): diagnostic efficiency, sensitivity specificity, positive and negative predictive values: 90%, 87·5%, 100%, 100% and 54·6%). (See also Supplementary Material, Appendix 8, Tables 8-1 to 8-3, Figure 8-1).

Table 2
Frequency of adverse events following vaccination

	Subjects recovered from mild COVID-19	History of PCR-positive asymptomatic COVID-19	Past subclinical infection detected by viral-specific IgG	Total
N	11	10	9	30
Subjects with some AE	3 (27·3%)	4 (40·0%)	5 (55·6%)	12 (40·0%)
Solicited local AE				
Site pain	0 (0.0%)	2 (20·0%)	1 (11·1%)	3 (10·0%)
Redness	0 (0.0%)	1 (10·0%)	1 (11·1%)	2 (6·7%)
Solicited systemic AE				
General malaise	0 (0.0%)	1 (10·0%)	0 (0.0%)	1 (3·3%)
Fever	1 (9·1%)	0 (0.0%)	0 (0.0%)	1 (3·3%)
Unsolicited systemic AE				
High Blood Pressure	2 (18·2%)	2 (20·0%)	3 (33·3%)	7 23·3%)
Headache	0 (0.0%)	1 (10·0%)	1 (11·1%)	2 (6·7%
Chills	0 (0.0%)	0 (0.0%)	1 (11·1%)	1 (3·3%)
Dry Mouth	0 (0.0%)	0 (0.0%)	1 (11·1%)	1 (3·3%)
Migraine	0 (0.0%)	0 (0.0%)	1 (11·1%)	1 (3·3%
Sinus tachycardia	0 (0.0%)	0 (0.0%)	1 (11·1%)	1 (3·3%)
Number of AE per subject				
Average (SD)	0·3 ± 0·5	0·7 ± 1·0	1·1 ± 1·9	0·7 ± 1·2
Median (IQR)	0 ± 1	0 ± 1	1 ± 1	0 ± 1
Range	0-1	0-3	0-6	0-6
Subjects with some VAAE	0 (0.0%)	3 (30·0%)	3 (33·3%)	6 (20·0%)
Solicited local VAAE				
Site pain	0 (0.0%)	2 (20·0%)	1 (11·1%)	3 (10·0%)
Redness	0 (0.0%)	1 (10·0%)	1 (11·1%)	2 (6·7%)
Solicited systemic VAAE				
General malaise	0 (0.0%)	1 (10·0%)	0 (0.0%)	1 (3·3%)
Unsolicited systemic VAAE				
High Blood Pressure	0 (0.0%)	0 (0.0%)	1 (11·1%)	1 (3·3%)
Number of VAAE per subject				
Average (SD)	0·0 ± 0·0	0·4 ± 0·7	0·3 ± 0·5	0·2 ± 0·5
Median (IQR)	0 ± 0	0 ± 1	0 ± 1	0 ± 0
Range	0-0	0-2	0-1	0-2

Data are n (%) unless otherwise specified. Average (SD)=Average ± Standard Deviation. Median (IQR)=Median ± Interquartile Range. AE=Adverse Event. VAAE=Vaccine-Associated Adverse Event.
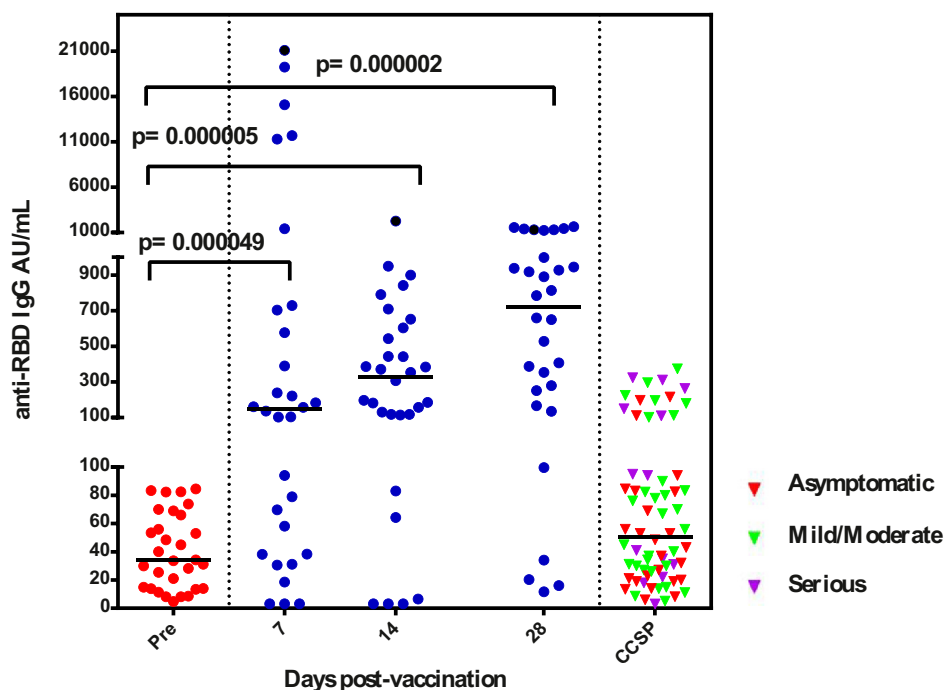
Figure 1. Anti-RBD IgG antibodies induced by a single dose of FINLAY-FR-1A vaccine in COVID-19 convalescents. Anti-RBD IgG concentration at days 0 (pre-vaccination), 7, 14 and 28 are expressed in arbitrary units/mL. CCSP: Cuban Convalescent Serum Panel.
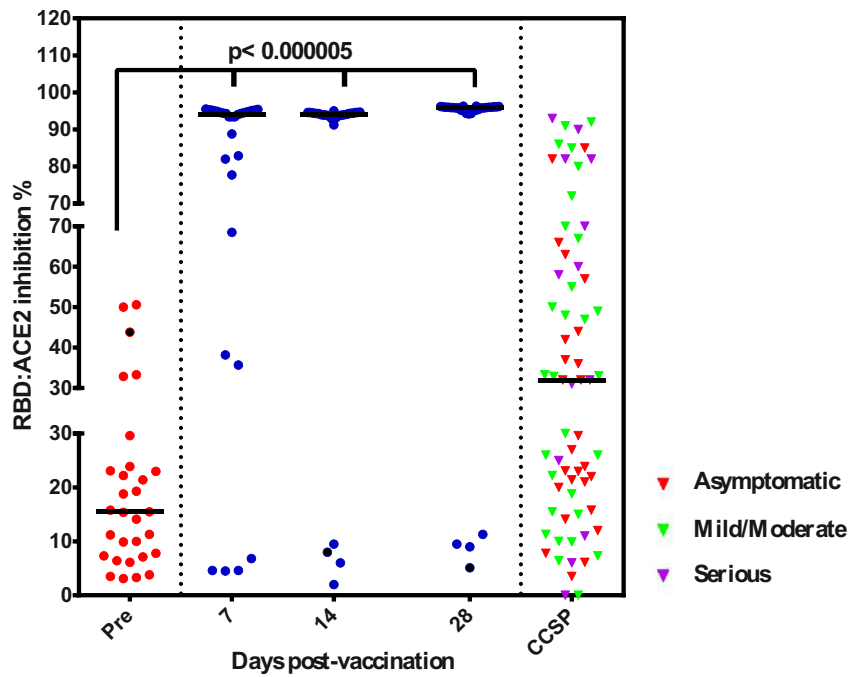
Figure 2. Capacity of anti-RBD IgG antibodies for inhibiting RBD:hACE2 interaction, as measured by competitive ELISA. % Inhibition of RBD:hACE2 interaction at 1/100 serum dilution at days 0 (pre-vaccination), 7, 14 and 28. CCSP: Cuban Convalescent Serum Panel.
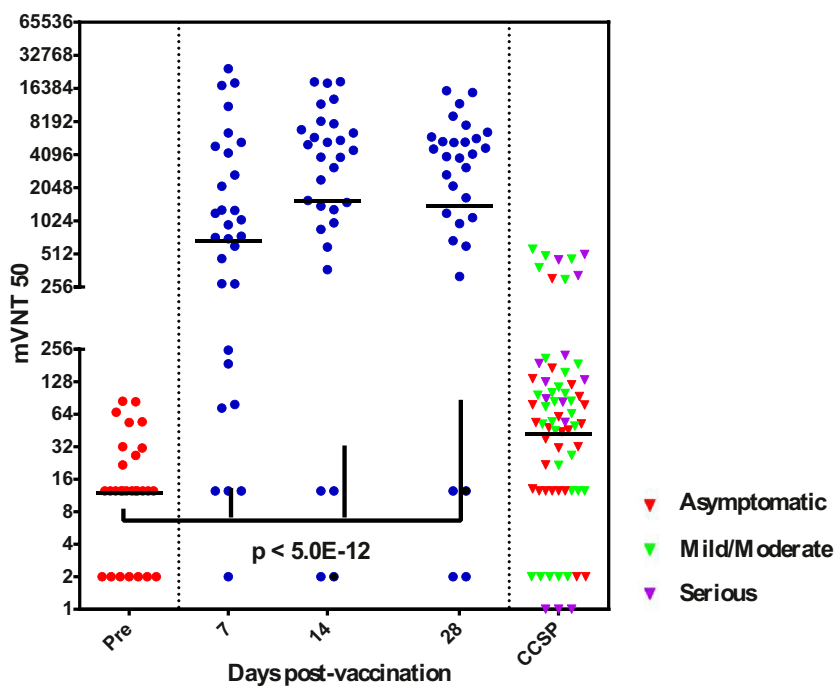

Figure 3. Half-maximal molecular virus neutralization titre inhibiting 50% of RBD:hACE2 interaction (mVNT$_{50}$), as measured by competitive ELISA at days 0 (pre-vaccination), 7, 14 and 28. CCSP: Cuban Convalescent Serum Panel.

4. Discussion

COVID-19 vaccines are being designed using several platforms; mRNA vaccines and viral vector vaccines are very immunogenic; there is concern regarding their reactogenicity [12, 19, 20]. Inactivated SARS-CoV-2 vaccines are less immunogenic; some reactogenicity has been reported [12, 21]. Recombinant spike protein vac-

cines are probably less immunogenic but provoke fewer adverse reactions [12, 18].

FINLAY-FR-1A is a vaccine based on recombinant d-RBD on aluminium hydroxide gel. It is being studied for the protection of naïve individuals, COVID-19 convalescent subjects (https://rpcec. sld.cu/en/trials/RPCEC00000349-En and this paper) and its use as a booster for persons already immunized with other vaccines is
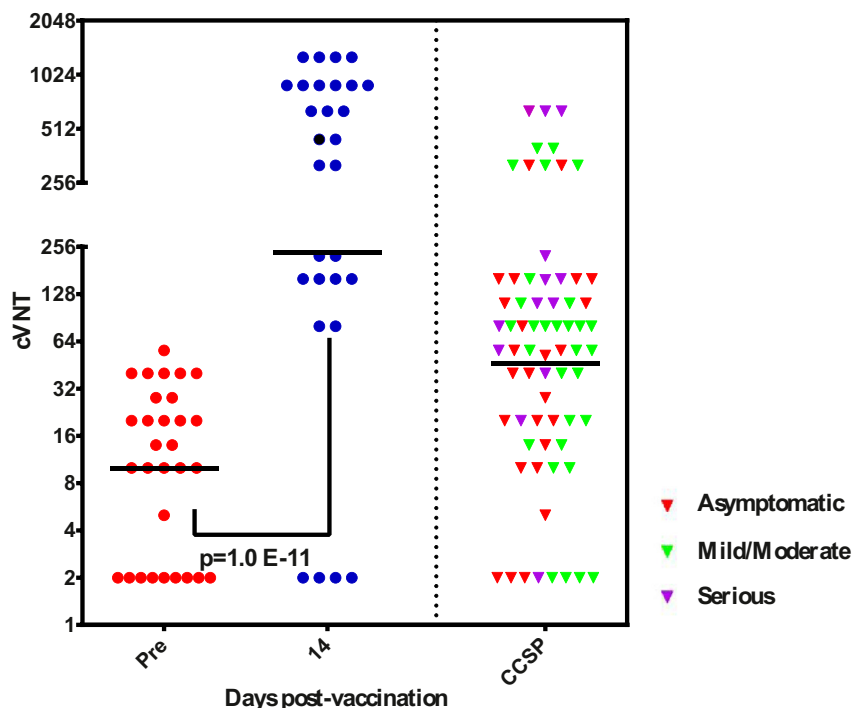
Figure 4. Conventional live-virus neutralization titre (cVNT) at days 0 (pre-vaccination), 7 and 14. CCSP: Cuban Convalescent Serum Panel.

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

	Pre-vaccination (day 0)	Days post-vaccination			CCSP
		7	14	28	
Anti-RBD IgG AU/mL					
median	34·0	146·6	330·4	722·2	50·8
25-75 percentile	14·0; 66·8	38·4;709·2	117·2; 615·3	2306; 1058·1	23·8; 94·0
RBD:hACE2 INH%					
Median	15·4	94·2	94·0	95·8	32·0
25-75 percentile	7·2; 23·3	75·4; 94·9	93·4; 94·4	94·8; 96·0	16·6; 62·2
mVNT$_{50}$					
GMT	21·7	817·4	2509·3	2243·2	59·3
95% CI	15·6; 30·2	366·8;1821·5	1234·9; 5098·7	1133·9; 4437·8	41·1; 85·5
cVNT					
GMT	9·9	N.A.	234·3	N.A.	46·4
95% CI	6·3; 15·4	N.A.	106·4; 515·8	N.A.	31·5; 68·4

AU/mL=anti-RBD IgG concentration expressed in arbitrary units/mL. RBD:hACE2 INH%= RBD:hACE2 inhibition % at a dilution 1/100. mVNT$_{50}$=serum dilution inhibiting 50% of RBD:hACE2 interaction. cVNT=conventional live-virus neutralization titre. GMT=Geometric Mean Titre. 95% CI=95% Confidence Interval. N.A.=not available. CCSP=Cuban convalescent serum panel.

being considered. It is safe: despite evaluating COVID-19 convalescents (including some with chronic disease, instead of healthy volunteers—as usual in clinical trials) we found fewer vaccine-associated adverse events than those reported in other studies [19-23].

This is the first phase I clinical trial in COVID-19 convalescents, and it was planned in subjects 19-59 years old. Ongoing study is being carried out in convalescents between 19-80 years old. Moderate or Severe COVID-19 were exclusion criteria by safety concern in this first study. Compensated chronic disease were included in the study. Only severe disease or decompensated chronic disease were excluded. (See Supplementary Material Appendix 2, Appendix 3).

A key concern is the nature, frequency, and severity of adverse events triggered by vaccination in COVID-19 convalescents.

Individuals seropositive to SARS-CoV-2 who received one dose of an mRNA vaccine had higher frequency of adverse events than seronegative individuals (75% of individuals had at least one event) [24]. Here, in a follow-up of adverse events over 28 days post-vaccination, only 6 individuals (20%) experienced vaccine-associated adverse events, with local events predominating over systemic.

The severe case of high blood pressure was classified as related to vaccination, both events were simultaneous, probably an anxiety-related reaction in a subject with a history of this disease. There is not any other reason to think the vaccine could cause high blood pressure.

In 26 out of 30 participants, a **s**ignificant increase in anti-RBD IgG was detected at day 7, showing stimulation of a secondary antibody response, as previously reported in healthcare workers
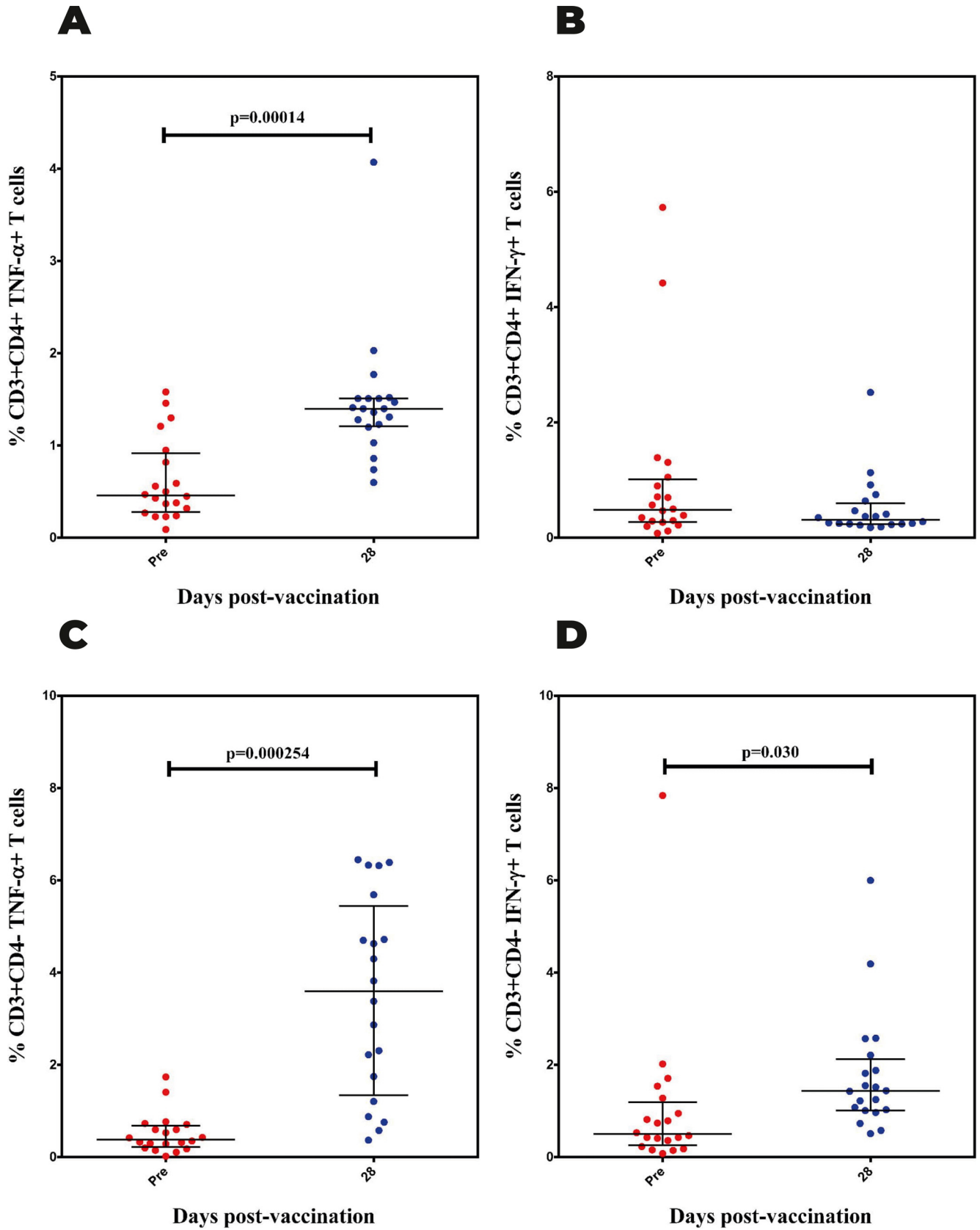
Figure 5. Frequency of RBD-specific T lymphocytes: (A) CD3+CD4+ TNFα^+, (B) CD3+CD4+ IFNγ^+, (C) CD3+CD4- TNFα^+, (D) CD3+CD4- IFNγ^+.

[24], and individuals who had been infected by SARS-CoV-2 [20, 25]. Anti-RBD IgG further had increased by day 14 and reached its highest value on day 28 (a 21-fold increase compared to pre-vaccination level and a 14-fold increase compared to CCSP); a similar finding was reported after one dose of mRNA vaccine BNT162b2 [25, 26].

All non-responders were women; they were the participants that elicited the lowest natural response to SARS-CoV-2 infection. These four women did not increase antibody levels after vaccination; however, two volunteers developed an adequate cellular immunity post-vaccination. As usual in other viral infections, a variable immune response has been described against SARS-CoV-2: [4, 6, 27, 28] some studies have shown a natural immunity against SARS-CoV-2 greater in women than in men, while no-differences associated to gender have been reported with COVID-19 vaccines [27-29].

The efficacy of anti-RBD antibodies in blocking the interaction between recombinant RBD and hACE2 as a primary indicator of functionality was evaluated in an inhibitory ELISA. We studied the quality of antibodies elicited by natural infection (before vaccination) and after vaccination. For all participants, the RBD:hACE2 inhibition ratio at a dilution of 1/100 was below 60% before vaccination; after vaccination, this ratio increased overtime for 26 out of 30 participants. All the responders attained a 94% inhibition ratio, 19 on day 7 and the remaining 7 on day 14 (the CCSP showed a median of 32% inhibition).

To evaluate the functionality of antibodies, the half-maximal molecular virus neutralization test ($mVNT_{50}$) is an important complement to the RBD:hACE2 inhibition ratio. The GMT of $mVNT_{50}$ on day 28 was notably higher than the pre-vaccination value and the CCSP value. The $mVNT_{50}$ is an excellent test for immunogenicity induced by COVID-19 vaccines. This is an in-vitro surrogate assay of the conventional neutralization test (cVNT) using live virus. The correlation between both tests has been verified, confirming that the in-vitro test could replace the complex cVNT.

The conventional virus neutralization test is considered the gold standard to evaluate neutralizing antibodies against SARS-CoV-2. Most individuals (80%) achieved cVNT>1/160, considered as protective levels, a value higher than that reported in other clinical trials [21, 22, 23].

Before vaccination, the four non-responder women had low levels of functional specific antibodies, and specific T-cell responses so the vaccine dose was just like a priming shot, at least for humoral immunity.

The delay of vaccination beyond the two-months after PCR negative result could be either unfavourable due to the decline of activated B cells, or could improve the immune response because lower levels of RBD inhibitory antibodies could prevent RBD clearance. The role of antibody concentration at baseline will be studied in next trials.

The frequency of specific T cells induced by the FINLAY-FR-1A vaccine is similar to that reported by other vaccines against SARS-CoV-2 [18, 26]. FINLAY-FR-1A increased the frequency of effector T cells producing TNF-α and IFN-γ, demonstrating induction of cellular immunity. RBD-specific IFN-γ CD4+ T cell subsets were identified on day 0 (before vaccination) and did not increase after vaccination; however, the strong booster effect of a single dose of the vaccine candidate suggests a T cell-dependent immune response, which requires T cell collaboration with B cells to produce specific IgG antibodies.

T-cell response plays an important role in COVID-19 mitigation, even in the absence of a measurable humoral response, as seen in non-responders. CD4+ T cells not only collaborate with B cells; they are also effector cells, producing IFN-γ, TNF-α and other cytokines [30, 31]. TNF-α is produced by effector T cells and innate cells, and can kill infected cells. Also, cellular response enhances T cell proliferation, cytokine production and contributes to T cell survival. IFN-γ is a key mediator of cellular immunity and enhances antiviral effects of cytotoxic T cells [30, 31].

Prevention of infection is related to the induction of specific functional antibodies, especially antibodies neutralizing the RBD:hACE2 interaction. However, CD4+ T cells are key to B-cell help and cytokine production, and to the cellular immune response. Particularly, cytotoxic T cells are necessary to eliminate an established infection. Both branches of the immune system are important to control COVID-19. The cellular immunity induced by vaccination complements the humoral response.

This study demonstrated the safety and immunogenicity of the FINLAY-FR-1A vaccine, eliciting high levels of neutralizing antibodies. B-cells were successfully stimulated 8 months on average after hospital discharge or serological diagnosis, demonstrating that natural infection leads to the production of long-term memory B cells, and that a booster dose induces a secondary immune response with IgG anti-RBD titres rapidly increasing by 7 days; memory T cells were also stimulated, and vaccination induced T-cell immunity to SARS-CoV-2. Our results are in accordance with a recent prepublished article, reporting that mRNA vaccines boost the immune response to SARS-CoV-2 one year after infection, and B cell clones express potent antibodies, which could cover some circulating variants [33].

While some studies report protective natural immunity induced by SARS-CoV-2, others evidence reinfection [8, 9, 26, 32]. A recent study in England has shown 7·6 reinfections x 100,000 person-days and previous infection with SARS-CoV-2 was associated with 84% reduction in the infection risk [34]. This reinfection rate should not be underestimated and the impact of new circulating strains on reinfection still needs evaluation. How efficient and long-lasting is the immune response elicited after viral infection is still under scrutiny; it can be foreseen that, due to insufficient natural protection, the vaccination of previously infected individuals could be necessary. This phase I clinical trial backed the use of a single dose of FINLAY-FR-1A for enhancing natural protection to SARS-CoV-2 and paved the way for the ongoing phase II clinical trial (Cuban Public Registry of Clinical Trials: RPCEC00000366-En, included in WHO International Clinical Trials Registry Platform).

Contributors

Declaration of Interests

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Data sharing

Role of the funding source

Acknowledgment

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lana.2021.100079.

References

[1] World Health Organization WHO Coronavirus (COVID-19) Dashboard, Geneva: WHO; 2021. Available from https://covid19.who.int (accessed, August 14, 2021).

[2] Shi-Lee W, Wheatley AK, Kent SJ, DeKosky BJ. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. Nat. Microbiol. 2020;5:1185–91.

[3] Arvin AM, Fink K, Schmid MA, et al. A perspective on potential antibody-dependent enhancement of SARS-CoV-2. Nature 2020;584:353–63.

[4] Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Zh Xing. Immunological considerations for COVID-19 vaccine strategies. Nat. Rev. Immunol. 2020 Available fromhttps://doi.org/. doi:10.1038/s41577-020-00434-6.

[5] Wu SL, Mertens AN, Crider YS, et al. Substantial underestimation of SARS-CoV-2 infection in the United States. Nat. Commun. 2020;11:4507 Available fromhttps://doi.org/. doi:10.1038/s41467-020-18272-4.

[6] International Society for Infectious DiseasesEstimation of the asymptomatic ratio of novel coronavirus infections (COVID-19). Int. J. Infect. Dis. 2020;94:154–5.

[7] Salazar E, Kuchipudi SV, Christensen PA, et al. Relationship between anti-spike protein antibody titers and SARS-CoV-2 in vitro virus neutralization in convalescent plasma. bioRxiv preprint. 2020: Available from: https://doi.org/ 10.1101/2020.06.08.138990

[8] Brouwer PhJM, Caniels TG, van der Straten K, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science 2020;369:643–50.

[9] Hotez PJ, Corry DB, Strych U, Bottazzi ME. COVID-19 vaccines: neutralizing antibodies and the alum advantage. Nat. Rev. Immunol. 2020 Available fromhttps://doi.org/. doi:10.1038/S41577-020-0358-6.

[10] Shen Ch, Wang Zh, Zhao F, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA 2020;27 Available fromhttps://doi.org/10.100/jama.2020.4783.

[11] Yang J, Wang W, Chen Z, et al. A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. Nature 2020;29 Available fromhttps://doi.org/. doi:10.1038/s41586-020-2599-8.

[12] Alturki SO, Alturki-Sawsan O, Connors J, et al. The 2020 Pandemic: Current SARS-CoV-2 Vaccine Development. Front. Immunol. 2020;11 Available fromhttps://doi.org/. doi:10.3389/fimmu.2020.01880.

[13] Ministerio de Salud Pública. Protocolo de actuación nacional para la COVID-19. Versión 1.5. La Habana; MINSAP: 2020. (Spanish).

[14] World Health Organization Causality Assessment of an Adverse Event Following Immunization (AEFI). 2nd Edition. Geneva: WHO; 2018.

[15] Plikaytis BD, Carlone GM. Program ELISA for Windows Userś Manual, Atlanta, GA, USA: Centers for Disease Control and Prevention; 2005. Version 2.0.

[16] Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nat. Biotechnol. 2020;38:1073–8.

[17] Manenti A, Maggetti M, Casa E, et al. Evaluation of SARS-CoV-2 neutralizing antibodies using a CPE-based colorimetric live virus micro-neutralization assay in human serum samples. J. Med. Virol. 2020;92(10):2096–104 Available fromhttps://doi.org/. doi:10.1002/jmv.25986.

[18] Keech C, Albert G, Cho I, et al. Phase 1–2 Trial of a SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine. NEJM 2020 Available fromhttps://doi.org/. doi:10.1056/NEJMoa2026920.

[19] Funk CD, Laferrière C, Ardakani A. Target Product Profile Analysis of COVID-19 Vaccines in Phase III Clinical Trials and Beyond: An Early 2021 Perspective. Viruses 2021;13:418 Available fromhttps://doi.org/. doi:10.3390/v13030418.

[20] Mathioudakis AG, Ghrew M, Ustianowski A, et al. Self-reported real-world safety and reactogenicity of COVID-19 vaccines: An international vaccine-recipient survey. medRxiv. the Preprint Server for Health Sciences. 2021. Available from: https://doi.org/ 10.1101/2021.02.26.21252096

[21] Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:181–92 Available fromhttps://doi.org/. doi:10.1016/S1473-3099(20)30843-4.

[22] Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 2020;396:467–78 Available fromhttps://doi.org/. doi:10.1016/S0140-6736(20)31604-4.

[23] Zhu FC, Li YH, Guan XH, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet 2020;395:1845–54 Available fromhttps://doi.org/. doi:10.1016/S0140-6736(20)31208-3.

[24] Saadat S, Rikhtegaran-Tehrani Z, Logue J, et al. Single Dose Vaccination in Healthcare Workers Previously Infected with SARS-CoV-2. medRxiv: the Preprint Server for Health Sciences. 2021. Available from: https://doi.org/ 10.1101/2021.01.30.21250843

[25] Krammer F, Srivastava K, the PARIS team, Simon V. Robust spike antibody responses and increased reactogenicity in seropositive individuals after a single dose of SARS-CoV-2 mRNA vaccine. medRxiv: the Preprint Server for Health Sciences. 2021. Available from: https://doi.org/ 10.1101/2021.01.29.21250653

[26] Samanovic MI, Cornelius AR, Wilson JP, et al. Poor antigen-specific responses to the second BNT162b2 mRNA vaccine dose in SARS-CoV-2-experienced individuals. medRxiv: the Preprint Server for Health Sciences. 2021. Available from: https://doi.org/ 10.1101/2021.02.07.21251311

[27] Cao S, Gan Y, Wang C, et al. Post-lockdown SARS-CoV-2 nucleic acid screening in nearly ten million residents of Wuhan, China. Nat. Commun. 2020;11:5917 Available fromhttps://doi.org/. doi:10.1038/s41467-020-19802-w.

[28] Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat. Med. 2020;26:1200–4 Available fromhttps://doi.org/. doi:10.1038/s41591-020-0965-6.

[29] Logunov DY, Dolzhikova IV, Shcheblyakov DV, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomized controlled phase 3 trial in Russia. The Lancet. Published Online February 2, 2021. Available from: https://doi.org/ 10.1016/S0140-6736(21)00234-8

[30] Mehta AK, Gracias DT, Croft M. TNF activity and T cells. Cytokine 2018;101:14–18 Available fromhttps://doi.org/. doi:10.1016/j.cyto.2016.08.003.

[31] Bhat P, Leggatt G, Waterhouse N, Frazer IH. Interferon-γ derived from cytotoxic lymphocytes directly enhances their motility and cytotoxicity. Cell Death Dis 2017;8(6):e2836 Available fromhttps://doi.org/. doi:10.1038/cddis.2017.67.

[32] Hansen CH, Michlmayr D, Madeleine Gubbels S, Mølbak K, Ethelberg S. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. The Lancet 2021. Available from: https://doi.org/ 10.1016/S0140-6736(21)00575-4

[33] Wang Z, Muecksch F, Schaefer-Babajew D, et al. Vaccination boosts naturally enhanced neutralizing breadth to SARS-CoV-2 one year after infection. bioRxiv: The Preprint Repository for the Biological Sciences. 2021. Available from: https://doi.org/ 10.1101/2021.05.07.443175

[34] Hall VJ, Foulkes S, Charlett A, et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). The Lancet. Published Online April 9, 2021. Available from: https://doi.org/ 10.1016/S0140-6736(21)00675-9