CLINICAL TRIAL PROTOCOL

CLINICAL STUDY ABDALA – Phase 3

SARS-CoV-2 spike receptor-Binding Domain recombinAnt protein noveL vAccine

Phase 3, multicenter, randomized, double-blind, placebo-controlled clinical trial for adult evaluation of the efficacy, safety and immunogenicity of the CIGB-66 vaccine candidate against SARS-CoV-2.

Study code: IG/CIGB-66I/CVD19/2103

Version: 1.0

March 15, 2021

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SUMMARY

COVID-19 caused by SARS-CoV-2 poses an unprecedented challenge for health systems around the world. Vaccines are required to address this health problem. Multiple vaccine candidates are under clinical evaluation, in order to obtain safe and effective preventive vaccines that manage to control this scourge. A phase 3, multicenter, randomized (1:1), double-blind, placebo-controlled clinical trial will be conducted with the primary objective of assessing the efficacy, safety and immunogenicity of the CIGB-66 vaccine candidate based on recombinant RBD subunit, administered intramuscularly in the prevention of symptomatic disease from infection with SARS-CoV-2. 48,000 subjects, aged between 19 and 80 years, apparently healthy or with chronic controlled diseases, who respond to the call for the trial and agree to participate voluntarily, will be randomly assigned to one of two study groups: I - RBD (50 µg) + Aluminum hydroxide (0.30 mg) / 0.5 mL and II - Placebo + Aluminum hydroxide (0.30 mg) / 0.5 mL. The investigational product (CIGB-66 or placebo) will be administered intramuscularly: 0.5 mL in the deltoid region, following the immunization schedule 0 - 14 - 28 days. In the hypothesis it is expected that the group that receives the CIGB-66 vaccine candidate has a reduction of ≥60% of the risk of symptomatic COVID-19 compared to the placebo group, in correspondence with other regulatory agencies which establish that the lower limit of the confidence interval (of 95%) is greater than 30%, rejecting the null hypothesis H0 = VE ≤ 30%. The main variable will be the vaccine efficacy, measured by the number of symptomatic subjects of COVID-19. For efficacy, patients with RT-PCR positive to SARS-CoV-2 will be used, from 14 days after the 3rd dose of the investigational product and who present at least one major symptom or sign or two of the symptoms or minor signs of COVID-19. A total of 151 cases of COVID-19 will be required to detect a 60% reduction in the rate of infection risk, with two intermediate analyses planned in this study, which will be executed when approximately 35% and 70% of the total number of cases to be observed are reached. Active/passive monitoring of the biosafety profile will be performed by identifying/characterizing adverse events (secondary endpoint). In addition, other secondary endpoints to be assessed are: (a) prevention of mild, moderate and severe forms of COVID-19; b) seroconversion of SARS-CoV-2 anti-RBD IgG antibodies; c) geometric mean of anti-RBD IgG-specific antibody titers; d) inhibition of the interaction of RBD with its ACE2 receptor by ELISA and e) viral neutralization of SARS-CoV-2. These immunological variables will be assessed at onset and 56 ± 5 days.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS USED

Abs: Antibodies

DNA: Deoxyribonucleic acid

Ag: Antigen

Intention-to-treat analysis: Strategy to analyze data from a randomized and controlled trial. All participants are included in the arm to which they were allocated, whether they received or not (or completed) the intervention given to that arm. This analysis prevents bias caused by the loss of participants, which may disrupt the baseline equivalence established by randomization and which may reflect non-adherence to the protocol. (Taken from the glossary of Cochrane collaboration terms; version 4.2.5)

GCP: Good Clinical Practice: A standard for the design, conduct, performance, monitoring, auditing, recording, analysis and reporting of clinical studies that provides a guarantee that the data and results reported are credible and accurate, and that the data, rights, integrity and confidentiality of the subjects of the study are protected. (Taken from regulation 21 - 08: Requirements for authorization and modification of clinical trials. CECMED)

CECMED: Center for the State Control of Drugs, Equipment and Medical Devices.

SREC / ERC: Scientific Research Ethics Committee / Ethics and Review Committee.

CIGB: Center for Genetic Engineering and Biotechnology.

COVID-19: Disease caused by SARS-CoV-2 (*Coronavirus Disease 2019)*.

CTCAE: *Common Terminology Criteria for Adverse Events*

ELISA: Enzyme-linked immunosorbent assay.

Adverse Event: It any unfavorable medical incident that occurs in an individual who participates in a clinical trial because of the administration of a pharmaceutical product. That incident does not necessarily have a cause-effect relationship with treatment. An adverse event can therefore be an unfavorable or unexpected sign (including an abnormal laboratory finding, for instance), symptom or disease temporarily associated with the use of a medicinal product. (Taken from the Guidelines on Good Clinical Practice, CECMED, 2000)

Unexpected adverse event: Any adverse event in which specificity or severity is not consistent with the risk information described in the protocol or in the Investigator's Brochure (if any). It also refers to an adverse event that has not been previously observed.

ICH: International Conference on Harmonization.

MINSAP: Ministry of Public Health (*Spanish acronym*).

PCR-RT: Real-time polymerase chain reaction.

RBD: SARS-CoV-2 virus receptor-binding domain.

Adverse reaction: It refers to an adverse event that is considered causally related to the investigational product, including overdoses and interactions with other drugs. All noxious and unintended responses to a medicinal product related to any dose, must be considered adverse drug reaction. A well-accepted definition of an adverse drug reaction is found in the Technical Report of the WHO (Series No. 850, 1995) stating that: "an adverse reaction is a harmful and non-deliberate response to a pharmaceutical product that happens at doses normally used in man for prophylaxis, diagnosis, therapy, or for the modification of physiological function. In clinical trials, the damages caused by overdoses, abuse or dependence, and interactions with other products must be considered adverse reactions".

SARS-CoV-2: Severe acute respiratory syndrome by coronavirus 2 (SARS-CoV-2). Coronavirus disease 2019 (COVID-19) has been defined as an acute respiratory infection that potentially can lead to severe acute respiratory syndrome caused by coronavirus 2 (SARS-CoV-2), with a clinical spectrum ranging from a disease very similar to common cold to severe pneumonia and severe acute respiratory failure.

vs.: *versus.*

I. GENERAL INFORMATION

1.1. Title of the clinical trial: "Phase 3, multicenter, randomized, double-blind, placebo-controlled clinical trial for adult evaluation of the efficacy, safety and immunogenicity of the CIGB-66 vaccine candidate against SARS-CoV-2". ABDALA Clinical Study – Phase 3.

1.2. Code: IG/CIGB-66I/CVD19/2103

1.3. Sponsor

- Center for Genetic Engineering and Biotechnology (CIGB).
  Group of Biotechnological and Pharmaceutical Industries (BioCubaFarma).
  Ave. 31 e/ 158 y 190, Cubanacán, Playa, La Habana, Apartado 6162, C.P. 11600, Cuba.
  ☎ (53-7)-2716022; Fax (53-7)-2716070 / 2736008; Web: http://www.cigb.edu.cu

1.4. Main Clinical Site

- ✓ "Saturnino Lora" Provincial Hospital, Avenida de los Libertadores, Santiago de Cuba, C.P. 90100, Cuba.

1.5. Participant institutions

Clinical Site	
Santiago de Cuba:	**Guantánamo:**
"José Martí" Polyclinic.	"Emilio Daudinot" Polyclinic (Norte).
"Frank País" Polyclinic.	"Omar Ranedo" Polyclinic (Centro).
"Carlos J. Finlay" Polyclinic.	"Asdrúbal López" Polyclinic (Sur).
"Camilo Torres" Polyclinic.	"Mártires del 4 de Abril" Polyclinic (Caribe).
"Ramón López Peña" Polyclinic.	"4 de abril" Polyclinic (Este).
"28 de septiembre" Polyclinic.	
"30 de noviembre" Polyclinic.	**Bayamo, Granma:**
"Josué País" Polyclinic.	"Jimmy Hirzel" Polyclinic.
"Mario Muñoz" Polyclinic (El Caney).	"Bayamo Oeste" Polyclinic.
"Ernesto Guevara" Polyclinic (Boniato).	
"Luis Ramírez" Polyclinic (El Cobre).	
"Julian Grimau" Polyclinic.	
"Armando García" Polyclinic.	

1.6. Monitors

CIGB Clinical Research Direction, Havana, Cuba:

- PhD. Francisco Hernández Bernal *(Main Monitor)*: Doctor in Medical Sciences; Medical Doctor; 1st and 2nd Degree Specialist in Hygiene and Epidemiology; Master's Degree in Clinical Trials; Master's Degree in Environmental Health; Full Professor of the University of Medical Sciences of Havana; Senior Researcher.
- M.Sc. Yenima Martín Bauta: Bachelor of Pharmaceutical Sciences; Master's Degree in Research and Drug Development; Research Associate; Assistant Professor of UCM – Villa Clara.
- Dr. Joel Quintana Guerra: Doctor of Veterinary Medicine and Animal Husbandry.
- B. Sc. Klaudia Urrutia Pérez: Bachelor of Pharmaceutical Sciences.
- B. Sc. Karen Urrutia Pérez: Bachelor of Pharmaceutical Sciences.
- M.Sc. Karem M. Catasús Álvarez: Degree in Psychology; Master's Degree in Public Health; Research Candidate; Instructor.
- Dr. Idania Baladrón Castrillo: M.D.; 2nd Degree Specialist in Gynecology; Master in Clinical Trials; Assistant Professor and Researcher.
- Dr. Yaquelin Duncan Roberts: M.D; 1st Degree Specialist in Clinical Laboratory.
- B. Sc. Claudia Martínez Suárez: Bachelor of Pharmaceutical Sciences.

MINSAP, Cuba.

- Dr. Dayamí Soler Cano: M.D; 1st Degree Specialist in Pharmacology; Assistant Professor; Research Associate. Provincial Coordinator of Clinical Trials in Guantánamo, National Coordinating Center of Clinical Trials (CENCEC).
- M.Sc. Sanlia Landazuri Llago: Bachelor of Pharmaceutical Sciences; Master'Degree in Natural Medicine and Bioenergetics; Research Associate; Instructor. Provincial Coordinator of Clinical Trials in Santiago de Cuba, National Coordinating Center of Clinical Trials (CENCEC).
- Dr. José Guillermo Martínez Urbay: M.D.; 1st Degree Specialist in General Comprehensive Medicine, and 1st and 2nd Degree in Ophthalmology; Assistant Professor. "Arnaldo Milián Castro" University Hospital, Santa Clara, Villa Clara.
- Dr. Alejandro Batista Izquierdo: M.D.; 1st Degree Specialist in Neurology. "Lucía Iñiguez Landín" Clinical Surgical Hospital, Holguín.
- Dr. Nilde-Liz Vasallo Hernández: M.D.; 1st Degree Specialist in Coloproctology. "Mártires del 9 de Abril" General Teaching-Hospital, Sagüa la Grande, Villa Clara:
- Dr. Alexis Rodolfo Pupo Micó: M.D.; 1st Degree Specialist in Internal Medicine. "Lucía Iñiguez Landín" Clinical Surgical Hospital, Holguín.
- Dr. Ricardo Lorenzo Mora Betancourt: M.D.; 1st Degree Specialist in General Comprehensive Medicine. "Rubén Batista Rubio" Polyclinic, Cacocum Holguín.

1.7. Principal Clinical Investigator

- PhD. María del Carmen Ricardo Cobas: M.D.; 1st Degree Specialist in Internal Medicine and 2nd Degree Specialist in Intensive Care and Emergency Medicine; Master's Degree in Emergency Medicine and Primary Health Care; Assistant Professor. "Saturnino Lora" Provincial Hospital, Santiago de Cuba.

1.8. Consultant – CIGB, Havana

- PhD. Verena Lucila Muzio González: Doctor of Biological Sciences; Doctor of Medicine; 1st Degree Specialist in Immunology; Assistant Researcher. Clinical Research Director, CIGB, Havana.

1.9. Responsibles for the storage and supply of product - CIGB, Havana.

- M. Sc. José L. Rodríguez Reynoso: Bachelor of Pharmaceutical Sciences; Master's Degree in Technology and Drug Control, First-level Technologist. Head of Supplies Group.
- Tech. Grettel Melo Suárez: Intermediate Career in Computer Science; 1st Level Innovative Technician.
- M. Sc. Anabel Álvarez Acosta: Bachelor of Science in Chemistry; Master's Degree in Contemporary Biotechnology Trends; Assistant Researcher.
- Tech. Daniel Rodríguez Reinoso: 1st Level Innovative Technician.

1.10. Responsibles for data handling and statistical analysis

Institute of Cybernetics, Mathematics and Physics (ICIMAF):

- PhD. Jesús Eladio Sánchez García: Doctor in Mathematical Sciences (specialization in Mathematical Statistics); B.Sc. in Mathematics; Full Researcher.
- M. Sc. Cristina Olimpia Chávez Chong: Master's Degree in Mathematical Sciences (Mention in Probability and Statistics); Bachelor of Science in Mathematics; Aspiring Researcher.
- M. Sc. Jorge Luis Azor Hernández: Master's Degree in Mathematical Sciences (Mention in Probability and Statistics); Bachelor of Science in Mathematics; Aspiring Researcher.
- B. Sc. Rolando Selgas Lizano: Bachelor of Science in Mathematics; Specialist for Science, Technology and Environment.
- B. Sc. Ernesto Rodríguez Martínez: Bachelor of Science in Mathematics.

Clinical Research Direction, CIGB, Havana:

- Eng. Marel Alonso Valdés: Computer Science Engineer (Responsible for Data Management).

1.11. Participants in the immunological evaluations

For the Biomedical Research Direction – CIGB, Havana

- M. Sc. Gilda Lemos Pérez: Bachelor of Science in Biochemistry; Master's Degree of Science. Contemporary Biotechnology Trends; Mention in New Product Research; Assistant Researcher.
- Tech. Giselle Freyre Corrales: Industrial Chemistry Technician.
- Tech. Amalia Vázquez Arteaga: Computer Science Technician.
- B. Sc. Sheila Chávez Valdés: Bachelor of Science in Biochemistry.
- Tech. Edelgis Coizeau Rodríguez: Analytical Chemistry Technician.
- B. Sc. Lismary Ávila Díaz: Bachelor of Science in Biochemistry.
- Tech. Hany Lianet González Formental: Industrial Chemistry Technician.
- B. Sc. Ricardo Martínez Rosales: Bachelor of Science in Microbiology.
- Eng. Yahima Chacón Quintero: Chemical Engineer; Aspiring Researcher.
- PhD. Ana Cristina Campal Espinosa: Doctor of Veterinary Sciences; Assistan Researcher.
- PhD. Gerardo Enrique Guillén Nieto: Doctor of Biological Sciences and Master's Degree in Chemical Sciences; Researcher and Full Academician. Biomedical Research Director.

For the Immunoassays Center (CIE), Havana

- M. Sc. Ariel Palenzuela Díaz: Bachelor of Science in Biochemistry; Master's Degree of Biochemistry (Mention in Immunology); Research Associate; Level II Technologist.
- PhD. Irinia Y. Valdivia Álvarez: Doctor in Health Sciences; Bachelor of Science in Microbiology; Assistant Researcher; I Level Technologist.

For the Civil Defense Laboratory (LISIDA), Havana

- B. Sc. María Teresa Pérez Guevara: Bachelor of Science in Biochemistry. Adjunct Researcher.

1.12. Business Management - CIGB, Havana.

- PhD. Miladys Limonta Fernández (Business Manager): PhD in Technical Sciences; Chemical Engineer; Master's Degree in Biotechnological Processes; 1st Level Technologist; Assistant Professor.

1.13. Logistics assurance - CIGB Clinical Research Direction, Havana.

- B. Sc. Elizeth García Iglesias: Bachelor of Science in Mathematics. Head of the Management and Control Group.

1.14. Management and Organization Committee

- PhD. Francisco Hernández Bernal: Clinical Manager of the Abdala Study, CIGB.
- PhD. María del Carmen Ricardo Cobas: Principal Clinical Investigator, "Saturnino Lora" Hospital.
- M. Sc. Yenima Martín Bauta: Monitor of the Abdala Study, CIGB.
- M. Sc. José L. Rodríguez Reynoso: Head of Medical Supplies, CIGB.
- PhD. Verena L. Muzio González: Advisor, CIGB Clinical Research Director.

1.15. Participant researchers: See **Annex 1**

1.16. Ethics and Review Committee/Scientific Research Ethics Committee

The present clinical trial protocol will be submitted to the consideration and approval of a Centralized Ethics Committee appointed for this purpose, made up of members of the Ethics and Review Committee of the "Saturnino Lora" Provincial Hospital of Santiago de Cuba (main clinical site), expanded with members of the Research Ethics Committees of the Universities of Medical Sciences in the eastern region, with scope to the Health areas involved in the ABDALA - Phase 3 study. The approval opinion of this Committee (including the resolution of appointment of its members, with their respective professional qualifications) will be attached to the protocol that will be sent to the national drug regulatory agency (Center for State Control of Medicines, Equipment and Medical Devices - CECMED) for the authorization to start the study in the country.

1.17. Independent Data Monitoring Committee

For the purposes of this study, an Independent Data Monitoring Committee (IDMC) will function, made up of highly qualified specialists, external to the CIGB and the participating clinical sites. They will be summoned for the review of the intermediate analyses and will accompany in the evaluation of the primary information to make timely recommendations to the sponsor. This Committee will be made up of:

- ✓ PhD. Hector Lázaro Lara Fernández *(Responsible for the Committee)*: Doctor of Medical Sciences; Doctor of Medicine; 1st Degree Specialist in Hygiene and Epidemiology; Master's Degree in Pharmacoepidemiology. National Coordinating Center of Clinical Trials (CENCEC), Havana.
- ✓ PhD. Teresita de Jesús Montero González: Of Doctor in Medical Sciences; Doctor of Medicine; 2nd Degree Specialist in Pathological Anatomy; Master's Degree of Science in Higher Education "Luis Díaz Soto" Military Hospital, Havana.
- ✓ Dr. José de Jesús Rego Hernández: Doctor of Medicine; 2nd Degree Specialist in Internal Medicine; Master's Degree in Pharmacoepidemiology. "Dr. Salvador Allende" Clinical-Surgical Hospital, Havana.

- ✓ Dr. Beatriz Amat Valdés: Doctor of Medicine; 1st Degree Specialist in Immunology. "Luis Díaz Soto" Military Hospital, Havana.
- ✓ Dr. Mayté Robaina García: Doctor of Medicine; 1st Degree Specialist in Biostatistics. National Coordinating Center of Clinical Trials (CENCEC), Havana.
- ✓ M. Sc. Dianne Yurien Griñan Semaná: Bachelor of Pharmaceutical Sciences; Master's Degree in Traditional Natural Medicine; Diploma in Clinical Trials and Pharmacoepidemiology; Research Associate; Instructor. Provincial Coordinator of Clinical Trials in Santiago de Cuba / CENCEC.

II. INTRODUCTION

2.1. Main data on the problem in question and its context.

The global pandemic of the new 2019 coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started in Wuhan, China in December 2019, and from then on has spread throughout the world[1,2]. As of March 15, 2021, 186 countries report the disease with 121 million confirmed cases and 2.68 million deaths from COVID-19 (2.14% fatality). In Cuba, 63,725 positive cases and 380 deaths have been reported (0.6% fatality)[3].

This new *Betacoronavirus* is similar to the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV); based on its genetic proximity, it probably originated from bat-derived coronaviruses with spread through an unknown mammalian host intermediate to humans[1]. The SARS-CoV-2 viral genome is rapidly sequenced to allow diagnostic tests, epidemiological monitoring, and the development of prevention and therapeutic strategies[4].

The clinical spectrum of a SARS–CoV-2 infection ranges from the absence of symptoms (asymptomatic infection) or mild respiratory symptoms to severe acute respiratory illness and death. Initially, it manifests mainly as fever, but sometimes only chills and respiratory symptoms occur due to mild dry cough and gradual dyspnea, in addition to fatigue and even diarrhea. In severe cases, the disease can progress rapidly, causing acute respiratory distress syndrome (ARDS), pneumonia, septic shock, irreversible metabolic acidosis, multiple organ failure, and clotting disorders, among other complications. The prognosis varies from recovery in most cases to torpid evolution and death[2,5].

Proper management of COVID-19 requires a better understanding of the pathogenesis of the disease. Currently, there is no specific drug or vaccine with Regulatory Authorization for Coronavirus SARS-CoV-2 and none has been fully assessed for its safety and effectiveness.

Although protection and social isolation measures that have been adopted by many countries have resulted in their citizens not acquiring infection by SARS-CoV-2, paradoxically making these people more vulnerable when faced with new waves of infection[6,7,8]. Certain groups of people are considered high risk because they have greater morbidity and mortality, including the elderly and people with underlying diseases (high blood pressure, diabetes mellitus, heart disease, cancer, chronic obstructive pulmonary disease, among others).[6,9].

There is consensus that, as long as no safe and effective preventive vaccines are available for SARS-CoV-2, in sufficient quantities to implement comprehensive immunization programmes, the world will not return to normality[10].

Vaccines are urgently needed to mitigate the consequences of this pandemic and protect humanity from future epidemics caused by this virus. In this sense, clinical trials with multiple vaccine candidates, with accelerated designs and overlapping of the traditional phases of clinical research, are currently carried out worldwide, without breaching Good Clinical Practices (GCP)[11]. Obtaining safe and effective preventive vaccines, as well as implementing them with broad global coverage, would be the fastest and safest strategy to manage this terrible pandemic[10].

One fifth of the Cuban population is aged ≥ 60 years, according to the National Population Aging Survey; of these, 58% have high blood pressure, 16% have diabetes mellitus, low blood pressure and thyroid diseases, and 19% are cardiopathic. A whole of 86,000 persons endures, in a simultaneous way, three of these chronic illnesses, what raises the probabilities of dying in case of contracting COVID-19. In this context, it should be considered that in Cuba a part of its population is more vulnerable to a SARS-Cov-2 infection[12].

At the Center for Genetic Engineering and Biotechnology (CIGB) in Havana, work has been made on several vaccine candidates using platforms already known to this institution and also considering the state-of-the-art of research around COVID-19, especially the immunological aspects necessary for the development of vaccines against this infection. One of these vaccine candidates is CIGB-66 for intramuscular administration, which has as active principle the proper recombinant protein RBD (SARS-CoV-2 virus receptor-binding domain) obtained in the CIGB in *Pichia pastoris*, adjuvanted to alumina.

RBD Component: Recombinant protein of the SARS-CoV-2 virus receptor-binding domain. Rationale for the use of RBD as vaccine antigen.

The selection of RBD as an active principle was based on the state-of-the-art of newly acquired knowledge about the structure of coronavirus SARS-CoV-2 and especially on S-protein, or spike protein, which is projected on the surface as a common crown in this family of viruses and which is responsible for the name that identifies them. S-protein has a receptor-binding domain named RBD.

It is known that, in COVID-19 convalescent patients most of the neutralizing antibody response against SARS-CoV-2, as well as a considerable portion of the cell response against it, is directed against the spike protein (S)[13,14], being the ideal candidate for inclusion in a vaccine preparation against this disease, endorsed not only by the specialized literature on other coronaviruses such as SARS and MERS[15,16,17], but also by its inclusion as a vaccine antigen in the preparations that Astra-Zeneca, Pfizer, Moderna and other companies evaluate at the present time[18,19,20].

However, previous research with other pathogenic coronaviruses has implicated S in pathological immunoamplification phenomena, both in animal models and in the clinical setting[21] thus the use of the spike protein in an experimental vaccine is not without risk. Moreover, the spike protein represents a formidable challenge for expression in heterologous systems if one wishes to employ it in a subunit vaccine: it is a molecule of considerable size (141 kDa) whose active form is a trimer in prefusion conformation with 17-22 N-glycosylation sites and a variable number of O-glycosylation[22,23] sites, the conformation of which is maintained by 13 disulfide bridges[24,25]. In fact, there are no reports of heterologous expression of the spike in trimeric conformation using microbial systems (yeast, fungi or bacteria).

S-protein is responsible for the interaction of SARS-CoV-2 with ACE2, its receptor in human cells[25,26]. This interaction occurs through a separate structural domain in that protein, known as RBD (Receptor-Binding Domain), which ranges approximately from cysteine 336 to cysteine 525, with a mass of approximately 25 kDa[25]. Although the RBD has some mobility with respect to the rest of the protein (it is found in two positions, the “open” and “closed” positions, which are important for interaction with the receptor)[27], its structure is rigidly constrained by the presence of 4 disulfide bridges with a complex topology, and its N-terminal is N-glycosylated in asparagines 331 and 343[28].

Many of the antibodies against RBD block its interaction with ACE2, thus neutralizing the SARS-CoV-2; in fact, epitopes recognized by a large number of neutralizing antibodies directed against the spike protein are found precisely in RBD[29]. For this reason RBD has been recognized as a promising vaccine candidate not only against SARS-CoV-2, but against other coronaviruses such as SARS and MERS[15,16]; being the first antigen successfully assessed in clinics during the BioNTech/Pfizer vaccine development program[30]. According to previous studies with SARS-CoV-1, the probability that immunization with RBD induces immunopathogenic phenomena is low[17,31], and this molecule has been successfully expressed in a wide variety of systems ranging from mammalian cells (HEK-293, CHO) to yeasts such as *Pichia pastoris*[32]. Finally, the relatively rigid structure of RBD - stabilized by four disulfide bridges - confers a high thermotolerance to this antigen, which may confer an advantage to vaccines based on this molecule in environments where cold chain failures can reasonably be expected[33].

Although the most studied RBD variant in the literature is the one that goes from arginine 319 to phenylalanine 541, it leaves at the N-terminal a strand that in the context of the native structure of S is actually part of a beta-sheet, and at the C-terminal end it leaves a free cysteine that can potentially form multimers with other RBD molecules or catalyze the exchange of disulfide bridges, thus affecting the structure of the molecule and leading to the formation of aggregates and intermolecular lattices[34]. Extended variants such as 319-541 and related variants

(318-510, 319-591) also have the disadvantage of being potentially less heat-resistant and more sensitive to protease action than shorter RBD variants (such as 331-528 and 331-532) due to the additional flexibility they bring to the ends of the molecule[35].

With the intention of using a variant of RBD **a)** as compact as possible and **b)** without free cysteines, RBD 331-530 was chosen as the starting vaccine antigen. This variant extends five residues into the N-terminal starting from the first cysteine of the domain, and so include the two N-glycosylation sites of the N-terminal of the domain, which according to previous data obtained for the SARS-CoV-1 RBD, are important for the expression of this domain in microbial hosts[36] and by the C-terminal it extends four residues after the last cysteine because there are several examples in the literature in which the RBD is successfully expressed with this C-terminal in microbial hosts[29,33].

Summary of non-clinical experimentation for the development of the CIGB-66 vaccine candidate

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus) is the etiological agent of the disease known as Covid-19. This virus has caused the second viral pandemic of this century and is the third beta-coronavirus to emerge as a human pathogen in the last 18 years[37]. More than 43 million people infected and more than 1 million killed by Covid-19 explain the growing negative potential of the pandemic of Covid-19 for humanity. The resurgence of new cases of COVID-19 in countries and territories where transmission control measures were relaxed suggests that only immunity provided through vaccination programs would control the spread of the virus or the fatal course of the disease in risk groups. Therefore, there is an urgent demand for a specific vaccine against SARS-CoV-2. Several pharmaceutical companies, universities, research institutes and government organizations are working on programs to achieve this goal. Projects funded by the Governments of various countries have seen an unprecedented increase. This scenario allows many approaches and different platforms to be tested to develop specific immunity against SARS-CoV-2, thereby obtaining an effective vaccine candidate.

The development of neutralizing antibodies (NAb) against the causative agent of Covid-19 disease (i.e., SARS-CoV-2), represents a viable option to obtain a vaccine candidate. These have been effective against other viruses such as respiratory syncytial virus[38] and Ebola[39]. In the case of SARS-CoV-2 it is known that antibodies specific to S-protein (from the surface) may have neutralizing activity. S-protein binds to the molecule ACE-2 (human angiotensin 2 converting enzyme) that mediates the entry of the virus into target cells[40] through its receptor binding domain (RBD) located within the S1 subunit. For those antibodies that the RBD do not recognize or recognize it with low affinity, an inhibiting potency of the binding to ACE-2 lower and an incomplete neutralization of the entry of the virus is described. Thus, the neutralizing antibodies that directly compete for the binding to ACE-2 are clearly the most effective[41]. Consequently, RBD was targeted for the development of Covid vaccines by many of the

vaccine developers[42]. In fact, it is known that the NAb-specific response against RBD can generate a protective response *in vivo* in experimental animals challenged with the virus[43] and prevent *in vitro* infection to human cell lines[44]. In addition, the use of recombinant RBD-based vaccines induces a high neutralizing activity in several species of experimental animals, including mice, rabbits and macaques, having demonstrated in the latter a protection against the viral challenge[43,45].

Within the development strategy of a vaccine, the selection of the adjuvant is a key element in the development of the formulation. Although many experimental adjuvants have been described, aluminum salts (Al3+) are still the most commonly used in parenteral vaccines. This type of adjuvation may result, depending on the nature of the antigen, in the induction of efficient humoral and cellular responses in the clearance of the virus and inhibition of its entry into the host cell as has already been demonstrated for strategies with similar design for the SARS-Cov virus.[46]. Aluminium salts are among the safest adjuvants, having been shown that exposure to aluminum in vaccinated children, according to the recommendations for vaccination programs, is low and the body does not easily absorb it[47].

The strategy proposed for CIGB-66 includes the aluminium hydroxide adjuvation of a viral S-protein fragment of SARS-Cov-2 (CRBDH6) produced recombinantly with a high degree of purity. Considering this, it was decided to carry out a series of experiences in experimentation animals aimed at developing an anti-Covid-19 candidate based on the RBD that could be immunized by the parenteral route (i. e. intramuscular).

In the course of non-clinical experimentation for the CIGB-66 vaccine candidate, antigen CRBDH6 produced from the HEK or CHO cell supernatant and later from *P pastoris* was used as basis. For both variants of the protein, similar physical-chemical-biological characteristics were evidenced that support its use in an alternative way in the evaluation of immunization strategies in the development of a vaccine for SARSCov-2. In the studies, doses between 5 and 50 µg were used in BALB/c mice and in non-human primates, which were administered in two-immunization schedules with a bi-weekly interval, including a third dose in some cases. In all studies, ELISA-type assays were conducted to study the induction of an IgG-specific response for RBD using RBD-FcH coating (for mouse) or RBD-FcM coating (for primates). Likewise, the quality of this response was characterized by a competitive test with RBD-FcH sera by ACE-2-FcM (for mice) and RBD-FcM by ACE-2-FcH (for primates).

Evaluation of the adjuvant platform and immunization schedules for the generation of an immune response specific for the receptor-binding site (RBD) of SARS-CoV-2 S-protein in BALB/c mice. Use of RBD-FcM model antigen. (Trial Code: **CICUAL 20041**)

One of the fundamental milestones in the development of vaccines is the definition of routes of administration, schedules and adjuvants that promote the establishment of a more effective antibody response. It is usual to explore these topics with easily obtained model proteins and then proceed to validate them with the vaccine antigens. Our first study was directed to the analysis of routes and options for adjuvant using as antigen a recombinant RBD protein fused to the Fc fragment of a mouse IgG (RBD-FcM) produced in HEK 293 cells of human origin. This fusion is generally used to increase the stability of the proteins expressed in this cell line and also for their purification, being very useful for proteins whose biological activity is mediated by dimerization.

In order to study the generation of anti-RBD humoral response in the serum of immunized animals, inoculations were performed by a parenteral route (i.e., subcutaneous (s.c.). For this purpose, 6 female BALB/c mice aged 6-8 weeks were inoculated in each group (except group 10 which carried only 3 animals) by the s.c. route in a 0, 14 scheme, in a final volume of 100 µL, with the following groups: 1. RBD-FcM (20 µg) + HBcAg (5 µg); 2. RBD-FcM (5 µg) + HBcAg (5 µg); 3. RBD-FcM (5 µg) + HBcAg (5 µg), Aluminum hydroxide (ALOOH) at 1.4 mg/mL; 9. H6RBD (20 µg) and 10. RBD-FcM (20 µg). Recombinant protein H6RBD was used as a control of RBD protein with incorrect folding and was obtained in *E. coli*.

Results obtained 13 days after the first dose showed 100% seroconversion with a geometric mean (GM) of 5 286 UStd/mL titers (standard units) in group 3 immunized with RBD-FcM (5 mg) + HBcAg (5 µg) in ALOOH which was significantly higher than group 2 inoculated with the same unattended Ags which had only 50% seroconversion ($p < 0.0112$, non-paired t-test). Something similar was observed a week after the second inoculation (GM G3: 33524 USTD vs GM G2: 80.25 USTD, $p=0.0018$). This evidenced the importance of adjuvant in AlOOH by parenteral route (**Figure 1A**).

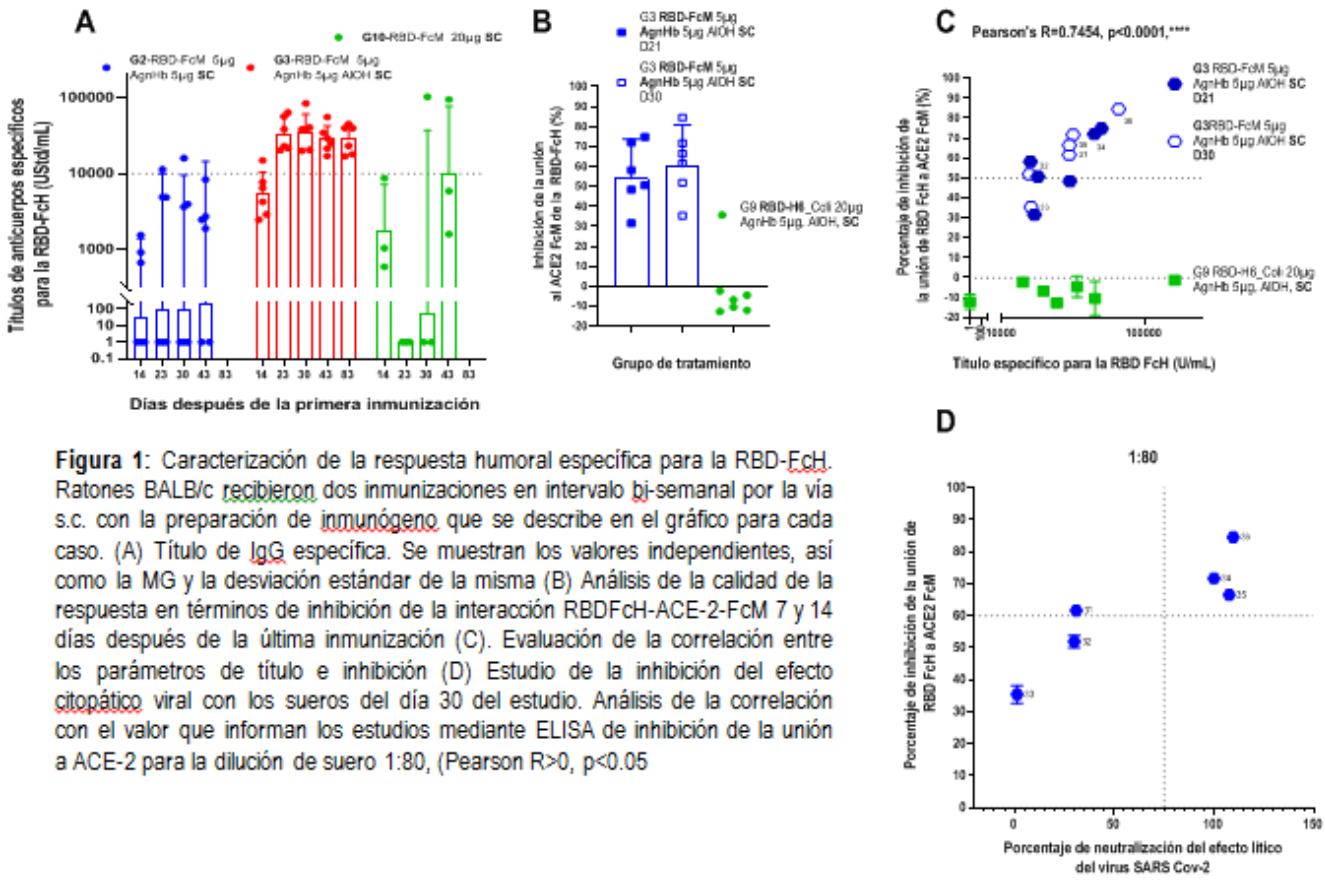


Figura 1: Caracterización de la respuesta humoral específica para la RBD-FcH. Ratones BALB/c recibieron dos inmunizaciones en intervalo bi-semanal por la vía s.c. con la preparación de inmunógeno que se describe en el gráfico para cada caso. (A) Título de IgG específica. Se muestran los valores independientes, así como la MG y la desviación estándar de la misma (B) Análisis de la calidad de la respuesta en términos de inhibición de la interacción RBDFcH-ACE-2-FcM 7 y 14 días después de la última inmunización (C). Evaluación de la correlación entre los parámetros de título e inhibición (D) Estudio de la inhibición del efecto citopático viral con los sueros del día 30 del estudio. Análisis de la correlación con el valor que informan los estudios mediante ELISA de inhibición de la unión a ACE-2 para la dilución de suero 1:80, (Pearson R>0, p<0.05

To verify the functionality of RBD-specific antibodies, sera were evaluated using an inhibition test for the RBD-binding to its receptor ACE-2 (SARS-CoV-2 virus receptor). Briefly, 1:200 diluted individual sera were incubated with an RBD-FcH molecule. This mixture was then added to a 96-well plate coated with the molecule ACE-2-FcM. After washing, the bound RBD-FcH molecule was detected with a human anti-Fc conjugated to peroxidase. Finally, after extensive washing of the plate, the reaction was developed by adding the TMB substrate. The inhibition percent was estimated as the fraction between the OD in the wells incubated with the sera and the wells where the RBD-ACE-2 interaction was maximum (serum-free) expressed in percent.

As a negative control in this study, serum from animals immunized with the H6RBD expressed and purified from *E. coli* was used to corroborate the need for proper folding of the antigen to obtain a quality response. Sera from group 3 (RBD-FcM (5 µg) + HBcAg (5 µg), ALOOH)) were evaluated in the inhibition test of the RBD-ACE-2 binding and they were compared to the response of the sera from the group 9 (H6RBD (20 µg)). As shown in **Figure 1A**, the animals receiving subcutaneous treatment (G3) showed the ability to inhibit RBD-ACE2 binding compared to the group immunized with the antigen variant obtained in *E. Coli* (Group 9), which did not develop

positive responses (**Figure 1B**). This result correlated with the titer of specific antibodies (R=0.745, p<0.0001) **(Figure 1C)**.

This result demonstrated the need to produce the vaccine antigen variants for DBR in a system that replicates the proper folding, so that antibodies with possible neutralizing capacity are induced. This inhibitory effect was also directly correlated with the inhibition of viral infection by SARSCov-2 in VERO-E6 cells (**Figure 1D**).

With this result we proceeded to analyze the immunogenicity of the antigen proposed to be part of the vaccine candidate: CRBDH6 either that obtained from higher cells (HEK-293 or CHO) or that produced from *Pichia pastoris*. In a first approach and taking into consideration previous studies of probable relationship of dose increase with the improvement of the quality of inhibitory response for other routes of administration (i.e. intranasal **CICUAL 20041**), a study was proposed where two dose levels of antigen CRBDH6_HEK were analyzed.

Evaluation of the effect of increased antigenic dose on immunogenicity of antigens representative of the receptor-binding site (RBD) of SARS-CoV-2 S-protein, produced in HEK-293, in BALB/c mice. (Trial Code: CICUAL/CIGB/20074)

In order to explore the immunogenicity of an RBD expressed in the same cell line as that used in previous experiments, but without the Fc murine region, an experiment was conducted in laboratory animals. This study also proposed the study of the effect of increased dose on the generation of humoral anti-RBD response in the serum of immunized animals. To this end, inoculations were carried out through the s.c. route with this antigen. 8 BALB/c female mice aged 9-10 weeks were inoculated in each group by s.c. route on a final volume of 100 µL or i.n. in a volume 25 µL, in a schedule 0, 14 days. Groups inoculated by s.c. route according to the immunogen were: 1) CRBDH6_HEK/ HBcAg (25/5µg) / ALOOH, 2) CRBDH6_HEK/ HBcAg (50 / 5 µg) / ALOOH, 5) CRBDH6_HEK (25 µg) / ALOOH, 6) CRBDH6_HEK (50 µg) / ALOOH, 7) HBcAg (2.5 µg) / ALOOH and HBcAg (2.5 µg) (i.n.) (negative control group. Adjuvation with ALOOH was performed at 1.4 mg/mL. Inoculated by both routes at each dose).

Comparative analysis of anti-RBD IgG-specific titers in serum of mice immunized by subcutaneous route between groups with equivalent amounts of RBD with and without HBcAg (G1: CRBDH6_HEK / HBcAg (25 / 5 µg) / ALOOH and G2: CRBDH6_HEK / HBcAg (50 / 5 µg) / ALOOH) and without HBcAg (5. CRBDH6_HEK (25 µg) / ALOOH and 6. CRBDH6_HEK (50 µg) / ALOOH) the formulation did not result in significant differences (**Figure 2A**). This result indicated that the addition of 5 µg of the HBcAg immunostimulator did not induce a differential response of IgG in serum compared to groups without it in the formulation. In order to better characterize this IgG response, the effect on the RBD-FcH binding to ACE2-FcM in solution was assessed (**Figure 2 B**).

The results of this analysis, one week after the second immunization, indicated that the increased dose of RBD (25 vs 50 µg), both in the presence and absence of HBcAg, caused a significant increase in the frequency of animals that develop an Ig response capable of inhibiting RBD-ACE2 interaction by more than 40% ($p<0.05$, Fisher's Test).

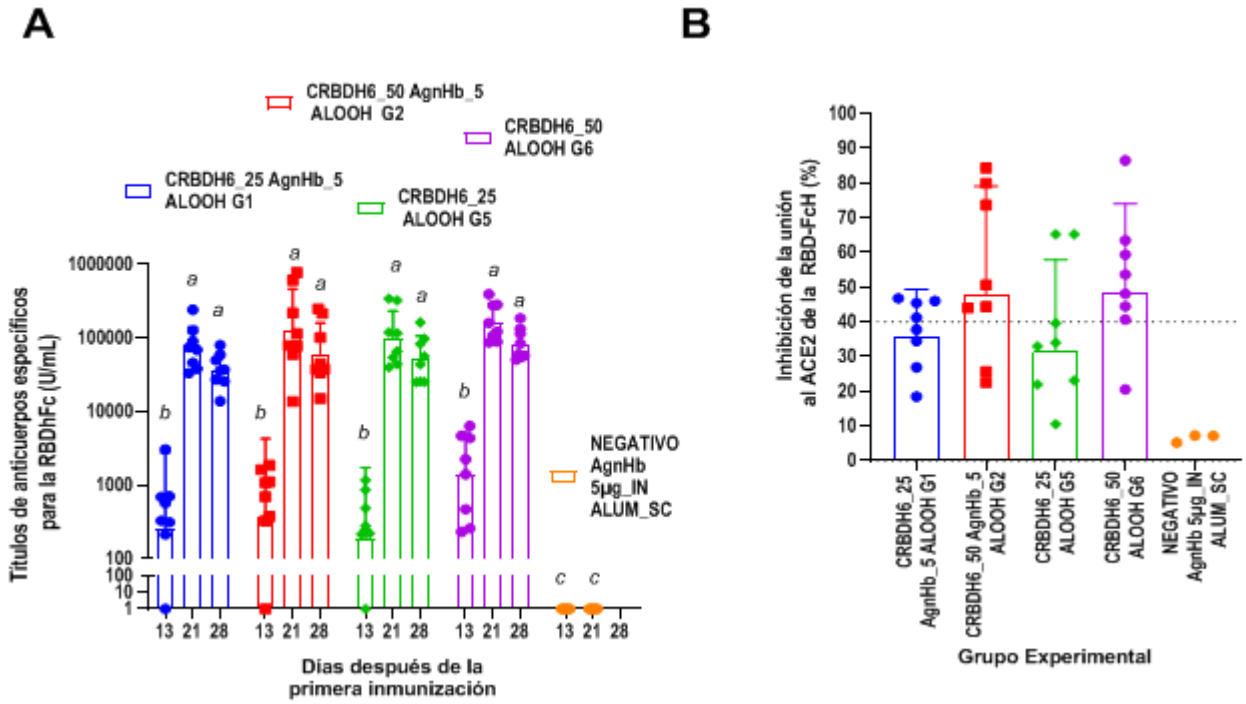


Figure 2. Anti-RBD-FcH IgG humoral response. BALB/c mice were twice immunized via s.c. with the immunogen preparation described in the figure for each case. **(A)** Studies of specific antibody titers. **(B)** Analysis of the effect of serum from immunized animals on the interaction of RBD-FcH with the ACE-2 FcM receptor. Independent data of each animal per group is shown in the form of points and the columns indicate the geometric mean.

Previous results indicated that the RBD protein produced in a human cell line is highly immunogenic when inoculated parenterally using aluminum hydroxide adjuvant. With only two doses, 100% seroconversion was obtained and the increase in antigenic concentration per dose led to a significant increase in inhibition of ACE-2 receptor binding.

Although the increased dose led to an increase in immunogenicity, analysis of data from the **CICUAL 20041** and **CICUAL 20074** studies, as well as recent literature on this type of subunit vaccine, indicated the existence of immunological reserves for the improvement of this response.[45,48]. One strategy to achieve this effect is to increase the number of administrations carried out. In order to study the effect of an additional dose, the study **CICUAL 20062** was conducted.

Study of humoral anti-RBD response in BALB/c mice after parenteral immunization (Trial Code: CICUAL 20062)

In order to study the generation of anti-RBD humoral response in the serum of immunized animals, inoculations were performed by a parenteral route (i.e., subcutaneous (s.c.) with the protein CRBDH6_HEK. For this purpose, 10 BALB/c female mice of 6-8 weeks were inoculated in each group on the subcutaneous route (s.c.) in a schedule 0, 14, 28, in a final volume of 300 µL, with the following groups: 1. Placebo; 2. RBD (20µg). Protein CRBDH6_HEK was adjuvated to ALOOH at 1 mg/mL. The Placebo group was prepared just like the rest of the immunogens; but without Ags. Ten days after 2nd and 3rd doses a positive response was observed in 100% of animals. IgG titers in the serum of individual mice after the 2nd dose showed a geometric mean of 1:6,492 that increased to 1:28 240 after the third dose. This resulted in a statistically significant increase (p = 0.0019, paired t-test) of more than 4 times between doses (**Figure 3**).

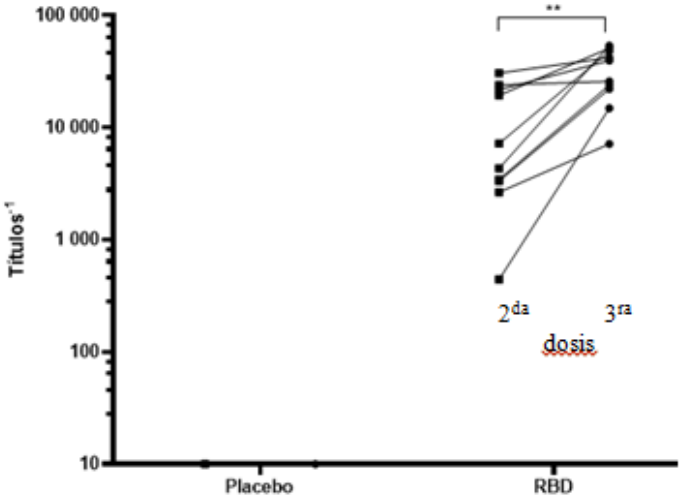


Figura 3. Respuesta humoral de IgG anti-RBD. Ratones BALB/c se inmunizaron tres veces por la vía s.c. con: 1. Placebo (AlOOH), 2. RBD (AlOOH). Los sueros se colectaron diez días después de cada dosis y los títulos se calcularon por ELISA. El incremento de los títulos entre 3ra y 2da dosis fue significativo (p= 0,0019, prueba t pareada)

Regarding the quality of the response, and similar to what has already been described, it was observed that after two doses, half of the animals achieved an inhibition percentage of RBD binding to ACE-2 higher than 50%. After a 3rd inoculation, all animals developed a serum immunoglobulin response capable of inhibiting the interaction by more than 50%, the arithmetic mean being 86.18%, significantly higher than that achieved with two doses (p= 0.0020; Wilcoxon test for paired samples). Additionally, the dispersion of values (i.e., standard deviation) was lower after the third dose (27.24 vs. 12.88) (**Figure 4**).

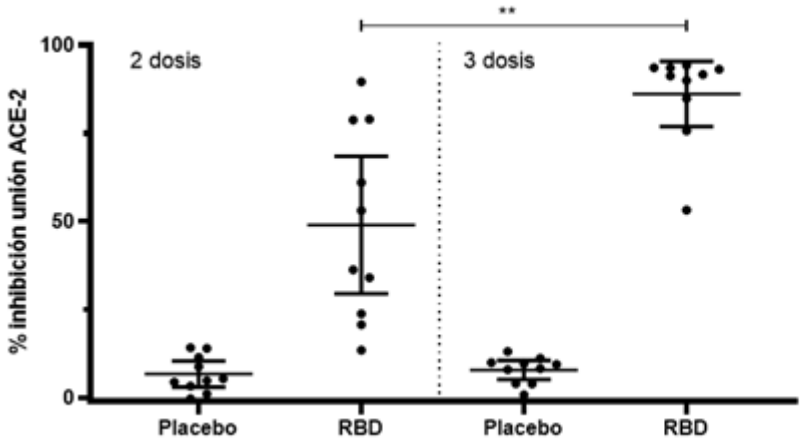


Figura 4. Ensayo de inhibición de la interacción RBD-ACE-2. Ratones Balb/c se inmunizaron dos y tres veces vía s.c. con: 1. Placebo (AlOOH), 2. RBD (AlOOH). Los sueros se colectaron diez días después de cada dosis y se diluyeron 1: 200 para la prueba. Con la prueba de Wilcoxon para muestras pareadas se demostró un incremento significativo luego de tercera dosis vs segunda dosis (p=0,0020). Cada punto se corresponde con un animal individual. Se representa la media ± IC 95%.

These results confirmed those previously obtained after the second dose and indicated that the addition of a third dose to the schedule significantly increases the levels of potentially effective response observed in the animals.

Study in Non-Human Primates

Multiple evidences from the clinical development of other candidates, as well as studies in non-human primates indicate that the course of the immune response differs greatly from that observed in rodents, both in magnitude and kinetics[45,48]. It was therefore considered appropriate to start two exploratory studies in non-human primates (Green monkey, Chlorocebus sabaeus) with antigen purified from both host types: *P. pastoris* (**CICUAL 20067**) and HEK/CHO (**CICUAL 20072**). The main objective of these trials is the qualitative and quantitative analysis of the safety of the dose to be administered in humans, and of the kinetics of the immune response in a species closer to humans. The studies were adjusted over time, in terms of dose frequency, to non-clinical observations in rodents.

The **CICUAL 20072** study analyzed the kinetics of the humoral immune response and the quality of the response in groups of two animals with an average age of 7 years. Two experimental groups were evaluated and included 2.-. Intramuscular administration of 500µL of 50µg of CRBDH6_HEK adjuvanted in ALOOH (0.286 mg/mL) including 5µg of HBcAg in the first two doses and in the re-activation dose; and 3. - Administration of 50µg of total CRBDH6_HEK divided between the two routes: a) 100µL by the intranasal route of 25µg of CRBDH6_HEK

accompanied by 2.5µg of HBcAg in the first two doses and 40µg of HBcAg at re-activation on day 35 of the study and b) 500µL by the intramuscular route of 25µg of CRBDH6 adjuvanted in ALOOH (0. 286 mg/mL) including 2.5µg of HBcAg in the first two doses and at re-activation.

IgG vs RBD-FcM		Animal_Experimental Group			
Time	Immunization	11405_2	11401_2	11413_3	11407_3
Day 0	X	425	1	1	1
Day 14	X	305	1	1	1
Day 21		527	309	3546	205
Day 28		829	563	11285	1231
Day 35	X	6738	3879	13549	4105
Day 42					

RBD-ACE-2 inhibition 1:200		Animal_Experimental Group			
Time	Immunization	11405_2	11401_2	11413_3	11407_3
Day 0	X	17.3	20.5	12.7	6
Day 14	X	15.35	11.3	8.95	1.65
Day 21		12.3	10.15	39	3.1
Day 28		8.9	12.2	50.35	7.5
Day 35	X	14.2	7.3	56.7	10.35
Day 42					

So far, effective seroconversion was observed at the systemic level in the four animals that received intramuscular doses, but a delay in the appearance of response is detected when compared to studies of the parenteral route in rodents. The neutralizing response reached 50% on day 28 of the study for only one animal (11413). This corresponds with studies of other vaccines in humans and with ongoing clinical studies for S-protein subunit variants or the RBD of SARSCOV-2, which indicate that optimal times between vaccinations are above 21 days and on average 28 days.[48,49].

The **CICUAL 20067** study analyzed the kinetics of the humoral immune response and the quality of response in groups of two animals with an average age of 2 years. Two experimental groups were evaluated and included recibieron tres Dose en el Schedule 0, 14, 28; out of:-. 2.-. Intramuscular administration of 500µL of 50µg of CRBDH6_PP adjuvant in ALOOH (0.286 mg/mL) including 5µg of HBcAg; and 3. - Administration of 50µg of total CRBDH6_PP divided between the two routes: a) 100µL by the intranasal route of 25µg of CRBDH6_HEK

accompanied by 2.5µg of the HBcAg in the first dose and 40µg of the HBcAg in the second and third doses and b) 500µL by the intramuscular route of 25µg of CRBDH6 adjuvant in ALOOH (0. 286 mg/mL) including 2.5µg of HBcAg.

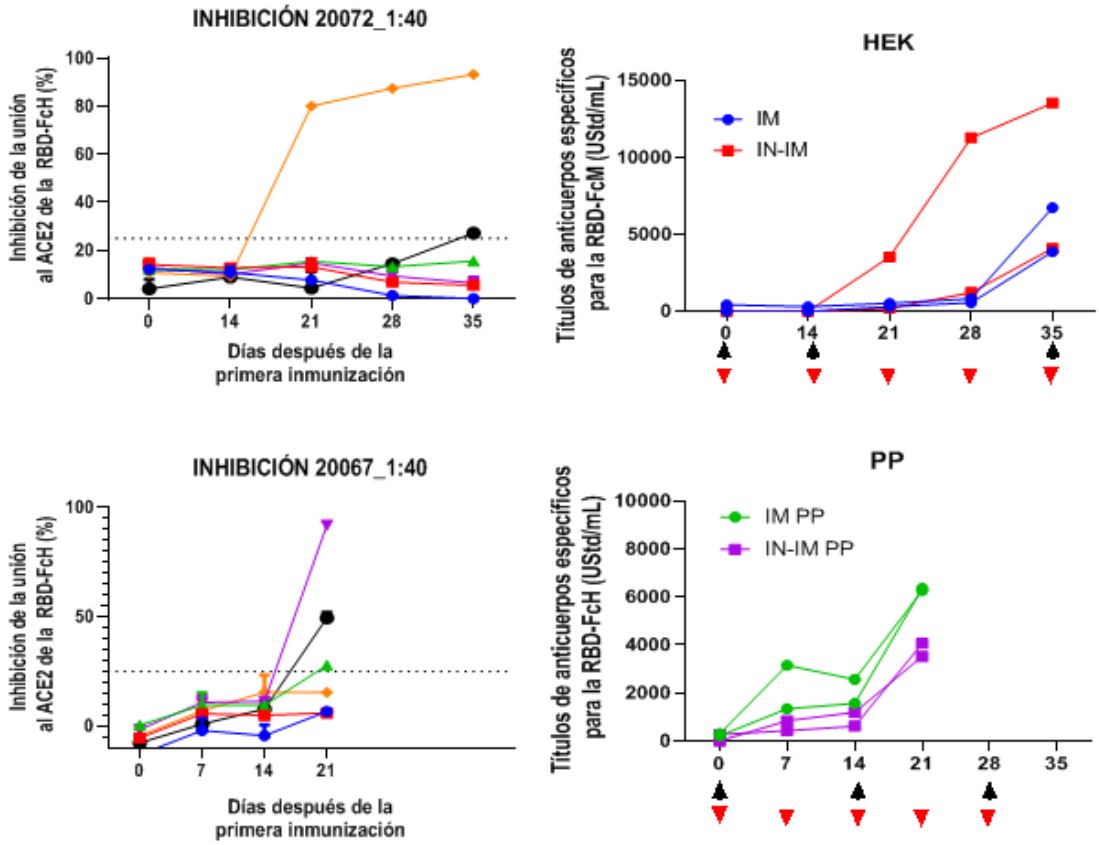
IgG vs RBD-FcM		Animal_Experimental Group			
Time	Immunization	**11531_2**	**11173_2**	**11175_3**	**11459_3**
Day 0	X	304.9	222.6	274.5	1
Day 7		3154.2	1346.9	432.2	842.87
Day 14	X	2568.6	1567.1	621.3	1191.8
Day 21		6270.4	6361.7	4070.6	3533.2
Day 28	X				

RBD-ACE-2 inhibition 1:200		Animal_Experimental Group			
Time	Immunization	**11531_2**	**11173_2**	**11175_3**	**11459_3**
Day 0	X	9.7	7.05	8.95	1.7
Day 7	X	4.65	9.95	11.45	6.8
Day 14		12.45	16.7	16.15	12.8
Day 21		7.1	43.3	8.9	14.55
Day 28	X				

The experimental evidence presented showed that CRBDH6 protein produced in human cells when adyuvated in ALOOH, and immunized by a parenteral route (i.e., s.c.), promotes serum IgG response at significant levels. This response is functional, and was shown to be able to block interaction with the SARS-CoV-2 virus receptor in an *in vitro* test. This inhibition test is known to predict quite reliably the neutralizing capacity of sera from experimental animals[44]. It is important to note that, although parenteral immunizations with the recombinant RBD protein were only performed by the s.c. route in mice, other groups have shown that response levels for this route and the intramuscular route are equivalent in this species[50]. In addition, results in non-human primates indicate that using the intramuscular route with CRBDH6 obtained in *P. pastoris* results in seroconversion of all animals studied 7 days after the second immunization.

Overall, the results obtained with different RBD variants evidenced that there is a dose-response effect (i.e., in terms of the amounts of RBD protein immunized), which is reflected as a significant increase in serum anti-RBD IgG titers and the ability to inhibit the RBD-ACE2 interaction. The same is true for the increase in immunizations in terms of the quality of the serum humoral response.

Finally, although HBcAg behaved as a good immunopotentiator for the nasal route when co-administered with CRBDH6, when administered by the parenteral route at doses of 5 µg, it did not contribute significantly to the increase in anti-RBD IgG-specific titers, nor to their quality understood as their ability to inhibit the RBD-ACE2 interaction. The studies developed so far in non-human primates show that, as has occurred for other vaccine candidates against viral entities, the response in this species, as in humans, requires longer intervals in the immunization schedules in order to establish an effective protective response.



So far, effective seroconversion was observed at the systemic level in the four animals that received intramuscular doses from seven days after the second immunization with similar kinetics to the non-human primate 11413_3 in the **CICUAL 20072** study. However, during this experimental time, neutralization of more than 40% was only observed in one animal. Although earlier increases in seroconversion were detected for the antigen obtained from *P. pastoris*, these results also point to the need for schedules with intervals longer than 14 days.

History in clinics

To date, about 15 clinical trials of vaccine candidates based on protein subunits to prevent COVID-19 are publicly registered. It is currently the most common platform for evaluation among vaccine candidates. Most of these vaccines contain the "S" protein of the complete SARS-CoV-2, or portions of it as RBD, in order to induce neutralizing antibodies as has been the case with most vaccines developed for SARS and MERS, which have different levels of efficacy[6,51,52,53].

One of these protein subunit candidates currently in Phase I/II clinical trial is Finlay-FR-1, sponsored by the Finlay Vaccine Institute (IFV-Spanish acronym). This candidate is based on the recombinant protein of the virus receptor-binding domain (RBD) with adjuvation. The study is stratified in two age groups, one 19-59 years old and one 60-80 years old and aims at evaluating the safety, reactogenicity and immunogenicity of the vaccine candidate FINLAY-FR-1 in 676 healthy volunteers, evaluating two dose levels: 10 µg and 20 µg, respectively, on a 0-28 day schedule, applied intramuscularly[52].

A phase I study in 50 volunteers between 18 and 59 years of age, sponsored by Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd evaluates a candidate with recombinant dimeric RBD protein (RBDDimer) expressed in CHO cells and adjuvanted, is developed in Chongqing, China. This multicenter, placebo-controlled study will follow-up volunteers for one year and is estimated to be completed by mid-2021. This same company sponsors a phase II clinical trial in Changsha, China, which will assess the conversion of neutralizing antibodies in 900 healthy volunteers between 18 and 59 years of age; and another phase I/II clinical trial in Hunan province, China to assess candidate tolerability and immunogenicity in 50 adults over 60 years of age[53].

On the other hand, Novavax sponsors three clinical trials with its vaccine candidate NVX-CoV2373 of protein subunits, in this case the recombinant nanoparticle of SARS-CoV-2 Peak Protein. The first is a multicenter phase I/II study in Australia and the United States to assess the safety and immunogenicity of this vaccine candidate with and without the adjuvant MATRIX-M™ in 1419 healthy volunteers between 18 and 84 years of age. Another phase II study with the candidate NVX-CoV2373 adjuvanted with MATRIX-M™ assesses the efficacy, immunogenicity and safety of 2904 adults between 18 and 84 years old with and without HIV infection. The third study is a phase III to assess the efficacy and safety of the candidate NVX-CoV2373 adjuvanted with MATRIX-M™ in 9000 volunteers from UK, serologically negative to SARS-CoV-2[53].

Other pharmaceutical companies have formed strategic alliances in obtaining SARS-CoV-2 vaccines, such as the joint sponsorship of Clover Biopharmaceuticals Inc./GSK/Dynavax. The Phase I trial assesses three dose levels (3,

9, or 30 µg), on 1-3 dose schedules of the Triune Peak Protein subunit candidate similar to native protein, with and without adjuvant (AS03 or CpG 1018 plus alumina) in healthy Australian volunteers[53]. Also the pharmaceutical company Sanofi Pasteur and GlaxoGuess Kline joined together for the development of vaccine candidate based on protein subunits, in this case the recombinant S-protein with and without adjuvant. This is a phase I/II clinical trial in 440 healthy adult volunteers in the United States, to be completed by the end of 2021[53].

Clinical experience with the CIGB-66 vaccine candidate

A Phase I-II, single-center, adaptive, randomized, parallel-group, double-blind, placebo-controlled clinical trial was conducted with the primary objective of assessing the safety and immunogenicity of the CIGB-66 vaccine candidate based on the recombinant RBD subunit, administered intramuscularly (in two immunization schedules), in the prevention of SARS-CoV-2 infection. In phase I, 132 volunteers participated and two strengths of the CIGB-66 vaccine candidate and two immunization schedules (0-14-28 and 0-28-56 days) were assessed, in a factorial design, which ended with an interim analysis for the selection of the two best experimental groups that fulfilled the hypothesis of the study for this stage (safety and seroconversion of anti-RBD IgG antibodies of SARS-CoV-2). The second stage involved 660 subjects aged 19 to 80 years, where the experimental groups selected in the first stage were compared with a placebo, with a hypothesis of 50% superiority in favor of the CIGB-66 vaccine candidate over the placebo in terms of seroconversion (primary endpoint). Active surveillance of the biosafety profile was performed by identifying/characterizing adverse events (also primary endpoint). As secondary endpoints during phase I (on days 0, 28, 42 and 56 for the short vaccination schedule; and 0, 28, 56 and 70 for the long schedule), the following were assessed: geometric mean of anti-RBD IgG-specific antibody titers, inhibition of the interaction of RBD with its ACE2 receptor by ELISA and humoral response of anti-RBD IgG-specific antibodies. These variables were considered during phase II, with the exception that the evaluation on day 28 was omitted.

The intermediate analysis of the results of the first phase showed a predominance of men and age ranged from 23 to 54 years, with similar means between groups, as well as for weight, height and BMI. Of the 132 subjects included, 127 (96.2%) completed the immunization schedule, showing high compliance.

In the analysis of adverse events for the short immunization schedule (0-14-28), at least one adverse event was reported in 29/66 included subjects, which corresponds to 43.9% of all those included. The frequency reported in the 25 µg and Placebo groups was the same (7 individuals out of 22 vaccinated, 31.8%), while in the 50 µg group it was 15/22 (68.2%), frequency greater than twice that reported in the first two. It can be expected with 95% confidence that in patients receiving the treatments administered to the 25 µg and Placebo groups, the proportion of patients with at least one event ranges from 14% to 55% while in those receiving treatment in the 50

μg group this frequency is greater (interval between 45-86%, p=0.020. The most frequently reported local events were: pain at injection site (16 events, 61.5%: 10 subjects in the 50 μg group), pruritus at injection site and induration, with two reports each (7.7%). Of the systemic adverse events, cephalea predominated with four 4 subjects (group 25 μg) accounting for 15.4%, high blood pressure and metallic taste occurring in three individuals each (11.5%).

Of the 52 events, all were mild or moderate with mild prevalence, with frequencies of 100%, 75%, and 88.5%, in the 25 μg, Placebo, and 50 μg groups, respectively. They had a possible, likely or definitive cause-effect relationship with vaccination 11, 5 and 13 events in the same order of groups. Definite and likely causality correspond to local adverse events, most of them mild (only two of moderate intensity), completely resolved. No serious adverse events were reported.

The probability of serious inadmissible toxicity (greater than 5%) was 0.31, i.e., less than 0.90.

For the long immunization schedule (0-28-56), during the 1st phase, the safety population consisted of 66 subjects vaccinated with at least one dose with this schedule. At least one adverse event was reported in 29/66 (43.9%) subjects. The highest frequency group was 25 μg, with 15 subjects of the 22 included, corresponding to 68.2%. The Placebo group reported the fewest subjects in only 22.7 out of 22 (22.7%); while in the 50 μg group there were 9 out of 22 (40.9%). It can be expected with 95% confidence that, with 25 μg treatment the proportion of patients with at least one event ranges from 45% to 86%; whereas in those receiving Placebo that frequency varies between 8-45% showing statistically significant differences, p=0.009. For the 50 μg group, it can be expected with 95% confidence to be greater than 18% and less than 64%.

The most frequent adverse events were: pain at injection site and cephalea; the first in 5 subjects in the 25 μg group and the same number in the Placebo group; while in the 50 μg it was reported in 6 subjects. Cephalea occurred in 6, 2, and 2 subjects in the 25 μg, Placebo, and 50 μg groups, respectively. Other less frequent events were: SARS-CoV-2 infection reported in 4 subjects (3 in the 25 μg group and one in Placebo). The event discomfort at injection site was observed in 3 subjects in the 25 μg group. The remaining adverse events were reported with a frequency of at most, 2 subjects per group.

Of the 69 events, almost all were of mild intensity, with frequencies of 83.3%, 100% and 95.0% in the 25 μg, placebo and 50 μg groups. One event was serious (high blood pressure), in the 25 μg group; but it was unrelated to the vaccine and corresponded to a subject with a history of that disease, which resolved with the measures taken. They had possible, likely or definitive cause-effect relationship with vaccination 9, 26 and 2 events in the

same order of groups. The adverse events with definitive and likely cause-effect relationship correspond to local events, mostly mild (only three of moderate intensity) and all were completely resolved.

The analysis of seroconversion of anti-SARS-CoV-2 anti-RBD IgG antibody considered seroconversion as that ≥ 4 times the initial antibody titer determination, at **days 28, 42 and 56** (for the short schedule) and **28, 56 and 70** (for the long schedule), with compared to the baseline time.

For the **short schedule** it can be stated with 95% confidence that for the 25 μg group seroconvert more than 64% of individuals from time 42 and the probability that the immune response is achieved in more than 50% of individuals is 0.99. While for the 50 μg group it is affirmed with 95% reliability that more than 72% seroconvert on day 42 and 85% on day 56; and the probability that the immune response is achieved in more than 50% of individuals is 0.99 since day 42. In the placebo group, no individual seroconverted.

Likewise, in the group that received the **short immunization schedule**, it can be stated that for the 25 μg group the geometric mean of anti-RBD IgG-specific antibody titers can be found with 95% confidence to be higher than 13.1 and lower than 38 also from day 42. On the other hand, in the 50 μg group the geometric mean of anti-RBD IgG-specific antibody titers can be found with 95% reliability greater than 71 and less than 244 on day 42; which increase slightly at day 56, where the lower limit is 83 and the upper limit is 291. In terms of percentage inhibition it is observed, in the **short schedule**, with 95% confidence that it is higher than 24% at day 42 and 25% at day 56 in the 25 μg group; while it is higher than 52% and 64% for the same respective times in the 50 μg group. The mean percent inhibition in the 50 μg group is higher than that in the 25 μg group; the confidence intervals are exclusive, (29.8;55.8) and (55.6;81.3) in the 25μg and 50 μg groups respectively. In addition, the percentage of subjects with seroconversion index was calculated ≥4 and more than 30% inhibited. In the 25 μg group it was 10 / 21 subjects (47.6%) and in the 50 μg group it was 19/21 subjects (90.5%).

In the case of the long immunization schedule, at the time of writing this protocol, immunogenicity tests were available up to day 56, i.e. one month after the second dose, and the test was pending at day 70 (14 days after the full immunization schedule), although the experimental results are already available.

From the data available from the risk-benefit analysis and the immunogenicity evaluation in the three groups receiving the long schedule, it is evident that there is no immunological response at day 28 and it begins to be observed at day 56 that individuals already have two doses of the vaccine candidate in the 25 μg and 50 μg groups, not so in the Placebo group, as expected. There is a dependence of the immune response on product strength, where it is shown that the 50 μg group has higher values of seroconversion, inhibition percentage,

neutralization and better Benefit-Risk ratio. These results indicate that the application of only two doses of vaccine, applied at one-month intervals, are not sufficient to achieve high levels of immune response in the majority of vaccinated subjects, making it necessary to apply the three doses of the complete immunization schedule.

2.2. Rationale for carrying out the study

COVID-19 caused by SARS-CoV-2 poses an unprecedented challenge for health systems around the world. Vaccines are needed to address this health problem, hence many clinical trials are currently underway with multiple vaccine candidates in order to obtain safe and effective preventive vaccines that manage to control this scourge. The CIGB has technological platforms and products that allow an accelerated transition towards the registration of a vaccine with high quality standards and in high quantities: **a)** experience in the use of the *Pichia pastoris* yeast as a model for the expression of recombinant proteins; **b)** having obtained in several expression systems, among them in CHO and *Pichia pastoris* yeast, a RBD (in the process of obtaining a patent).

Once demonstrated the safety and immunogenicity of CIGB-66 in preclinical and toxicological trials, in addition to its physico-chemical characterization and a high performance productive escalation that complies with GMP, the human evaluation of this vaccine candidate is appropriate.

The demonstration of efficacy of this vaccine candidate against SARS-CoV-2 infection will have an impact on pandemic control in Cuba and other countries. It would contribute to reduce the chains of transmission, avoid the torpid evolution of patients, with fatal outcome in many of them, in addition to the economic elements.

III. OBJECTIVES

3.1. General Objective

- ❖ Evaluate the efficacy, safety and immunogenicity of the intramuscularly administered CIGB-66 vaccine candidate in the prevention of symptomatic disease due to SARS-CoV-2 infection.

3.2. Specific objectives

- ✓ Demonstrate the efficacy of the CIGB-66 vaccine candidate in the prevention of symptomatic COVID-19, according to its intensity, in individuals confirmed by RCP to SARS-Co-V-2, after administration of the complete vaccination schedule.
- ✓ Assess immunogenicity of CIGB-66 vaccine candidate in terms of seroconversion of anti-RBD IgG antibodies of the SARS-CoV-2 and inhibition response of interaction of RBD with its ACE2 receptor and viral neutralization, in a subgroup of participants.
- ✓ Identify and describe adverse events that may occur after intramuscular administration of the CIGB-66 vaccine candidate in all subjects during the trial execution period.

3.3. Working hypothesis

In the group receiving the vaccine candidate CIGB-66 administered by the intramuscular route, in a three-dose schedule (0-14-28 days), a ≥60% reduction in the risk of symptomatic COVID-19 with respect to the placebo group is expected, in correspondence with international requirements that the lower limit of the confidence interval (95%) be greater than 30%, rejecting the null hypothesis $H_0 = VE \leq 30\%$.

IV. MEDICAL DEONTOLOGY

4.1. Ethics and Review Committee/Scientific Research Ethics Committee (SREC)

To start the conducting the clinical trial protocol it will be necessary to obtain the opinion of the Centralized Ethics Committee, which will certify after the corresponding evaluation and analysis that the document:

- ✓ Complies with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, adopted by the World Medical Assembly, Fortaleza, Brazil, 2013). The study will be published in the Cuban Public Record of Clinical Trials (WHO primary record) before inclusion of the first individual.
- ✓ It conforms to the ethical standards and criteria established in the national and international codes of ethics and legal regulations in force in Cuba (Good Clinical Practice Guidelines, CECMED 2000, Cuba; Good Clinical Practice Guide of the International Conference on Harmonization - ICH E-6 (R2)).
- ✓ It includes the form of protection of the rights and welfare of the subjects involved.
- ✓ Meets the standards and minimum requirements established for obtaining consent from the patients.
- ✓ Satisfactorily describes the illegibility criteria of patients.

4.2. Ethical aspects in the trial conduct.

The clinical trial was duly endorsed from the ethical point of view for the following reasons:

a. The clinical trial is approved by Scientific Research Ethics Committees and by the Cuban regulatory agency of medicines (CECMED). Likewise, as a contribution to research transparency, the protocol will be visible in the Cuban Public Registry of Clinical Trials. Available from: http://registroclinico.sld.cu
b. In toxicity studies in animals no undesirable manifestations of the CIGB-66 vaccine candidate were found in the inoculation site nor at systemic level.
c. A risk-benefit balance supported this research to demonstrate the efficacy of the vaccine candidate in the prevention of symptomatic disease by SARS-CoV-2. Exploratory studies conducted (phase I-II) in about 800 individuals show, in terms of safety and immunogenicity, that the benefits are far greater than the risks, with the possibility that participants enjoy the best possible quality of life, with some level of protection against the current COVID-19 pandemic. There have been no serious adverse events attributable to the vaccine and, after having applied more than 1400 doses of CIGB-66, adverse events have been minimal, of mild intensity, associated with the injection site and completely resolved without the need for any medication.
d. For this Phase 3, it is expected that the investigational product will have an adequate safety profile, as observed in the previous study; however, in the event of the occurrence of any adverse event, countermeasures will be taken, which may include definitive discontinuation (**Annex 15**). The management of

these medical contingencies will be carried out by qualified specialists, trained in the care of medical emergencies; for this purpose, each clinical site had a vital support area, with all the equipment, inputs and medications required to initially address any emergency, until the immediate transfer of the subject to the secondary level of care (coordinated SIUM for rapid action for the time the clinical trial requires it).

e. Participants will be asked for written consent, after knowing the possible inconveniences of using the product, the tests to be performed (their importance and usefulness), as well as the procedures necessary for the collection of biological samples.
f. The study will be randomized, double-blind and considers the use of placebo, which is recommended in international guidelines for prophylactic vaccine clinical trials. This type of design includes methodological pillars of scientific research (such as randomization; masking and control), which provide the study with the maximum rigor, scientific credibility and impact, according to Evidence-Based Medicine. The volunteers who will receive the placebo will have the possibility, if they accept, of being vaccinated once the efficacy of the CIGB-66 vaccine candidate had been demonstrated, being a commitment of the sponsor (CIGB).
g. All the care and evaluation of the subjects included in the study will be carried out by trained medical and paramedical personnel.
h. The integrity of the subjects will be respected, ensuring confidentiality of their personal data.
i. An Independent Data Monitoring Committee (IDMC) will be established to review analyses and safety issues. Its suggestions will be taken into account for decision making.
j. All clinical investigators will be asked to declare the absence of conflicts of interest.

4.3. Instructions for obtaining the informed consent.

For a patient to be included in the trial, written and signed informed consent shall be obtained **(Annex 4)**. During this process, all relevant information related to the study will be explained to the subject so that he/she can freely decide whether or not to participate. The individuals will be informed of the right to participate or not and to withdraw their consent at any time, without facing limitations for medical care or other retaliations.

Participants will have the necessary time to decide about their participation in the study. The protocol foresees that the physician provides the patient with the information sheet (**Annex 5**) so that he/she can consult at home with family and friends before making a decision.

The researcher must obtain the oral and written consent from the subject only after ensuring that he/she understood all the information provided. The procedure will be provided in a standard writing, in easily understandable language (it should not be technical, but practical). Neither the investigator nor the study personnel can influence the subject's decision to participate or continue in the study.

The information that will be provided to the individual must include at least the following:

- The study presupposes research.
- The objectives, immunizations, procedures to be followed, potential risks and benefits related to participation in the study, as well as any inconvenience that this may entail.
- Confidentiality of personal data and primary information generated in the study.
- The subject will know if new information becomes available that may be relevant to the decision to continue participating in the study.
- The expected duration of their participation in the test, as well as the frequency and type of evaluations.
- Voluntary participation in the study and the possibility of rejecting or abandoning the study without penalty or loss of the benefits to which they would have had access if they had not been included.

The subject will write (in black and white) his/her name, the date and time of granting the informed consent. Likewise, will write (in black and white) the name of the doctor who gave the relevant explanations; the latter will sign the document. A copy of the informed consent form will be given to the individual (the original form will be kept at the clinical site in the Reseracher's Folder). If the participant is unable to read or sign the document, an oral presentation will be made and the signature of his/her legal representative will be obtained, provided it is witnessed by a witness not involved in the study and mentioned in the same document. No individual can be included in the study without prior consent. The signing of the form by the subject does not release researchers, institutions or sponsors from their obligations due to negligence.

4.4. Ethical responsibilities of all participants in the research.

Researcher: Adherence to the procedures established in the protocol and informing and requesting the consent of the subjects.

Institution: Ensure proper maintenance and use of the facilities by the researcher and submit the protocol for approval by the Review and Ethics Committee (facilitated by the responsible researcher).

Research team: Ensure compliance with assigned responsibilities.

Sponsor: Guarantee the quality of the investigational product. Monitor the execution of the clinical trial protocol, verifying adherence by all researchers, and all ethical aspects contained therein.

Ethics and Review Committee: Review and approve the study protocol ensuring the protection of the rights, safety and welfare of the subjects involved in the study and provide public assurance of that protection. Verify the progress of the study and the adherence of the researchers to the protocol.

CECMED: Safeguarding the integrity of subjects through review, approval and monitoring of the trial.

GENERAL CONCEPT

5.1. Trial design

A phase 3, multicenter, randomized (1:1 ratio), double-blind, placebo-controlled clinical trial will be conducted with the primary objective of assessing the efficacy, safety and immunogenicity of the CIGB-66 vaccine candidate based on recombinant RBD subunit, administered intramuscularly in the prevention of symptomatic disease from infection with SARS-CoV-2.

A total of **48,000 individuals** will be included and randomly assigned to one of two study groups:

- ✓ Group I: RBD (50 µg) + Aluminum hydroxide (0.30 mg)/0.5 mL.
- ✓ Group II (Control): Placebo + Aluminum hydroxide (0.30 mg)/0.5 mL.

The investigational product (CIGB-66 or placebo) will be administered intramuscularly: 0.5 mL in the deltoid region, following the immunization schedule 0 - 14 - 28 days.

Because it is a double-blind study, neither the individual, the physician, the analysts, or the clinical trial monitors will know which group they were assigned to (the group will only be revealed at the end of the rigorous analyses provided for in the protocol).

The vaccination process will be completed at the clinical site (in several vaccination stations, by the nursing staff responsible for the activity) and the subjects will be on an outpatient basis.

Time 0 will be considered when the subject is included in the study (once the selection criteria are confirmed) and receives the first dose of the investigational product.

The medical team that conducts the study will be responsible for all the medical care that participants will receive, from the time they are included, for the duration of the clinical trial.

For the evaluation of efficacy (primary endpoint) all subjects will be considered **from 14 days after the 3rd dose of the investigational product**, who meet the "case definition" (specified in section 8.1.1. of the present protocol), which includes the virological diagnosis of SARS-CoV-2 by RT-PCR in addition to the presence of signs or symptoms of COVID-19. **A total of 151 COVID-19 cases will be required to detect a 60% reduction in the infection risk rate**, with two intermediate analyses planned in this study, to be run when approximately 35% and 70% of the total number of cases to be observed are reached.

All measures will be taken to ensure that the vaccination is carried out safely. For the safety assessment, there will be an active/passive surveillance of adverse events, considering that the research team will look for the appearance of any medical eventuality (mainly in the first 72 hours after the application of each dose of the investigational product) and at the same time, the participants in the study will be instructed on how to proceed in case of an adverse event, for its reporting and adequate medical attention. Thus, for each dose there will be an evaluation **prior** to product administration and within the **first hour after** product inoculation, in person at the clinical site (**Annexes 6** "*Vaccination Completion Consultation*" and **7** "*Medical Record of Adverse Events*"). Subsequently, with the support of medical students (in higher years) and family physicians in the family doctor's offices where the subjects are dispensed, 20-30% of those vaccinated in the Community will be visited each day (within 72 hours after each dose) with the intention of identifying and characterizing the adverse events that may occur (**Annex 8** "*Active screening for adverse events in the field*"). On the other hand, subjects will be instructed on the need to contact the ABDALA study research team in case of any medical eventuality. This monitoring will be carried out in the period between each dose (14 days). However, safety monitoring is foreseen throughout the duration of the trial, and even in the long-term outside the protocol (minimum up to one year) by their respective Health Areas (family physicians) where any Medical incidence that is of interest to the project (for example, if a pregnancy / delivery passed normally, etc). Observations at the clinical site include anamnesis, vital signs (temperature, blood pressure, respiratory and cardiac frequencies), inspection of the inoculation site (for the detection of local symptoms), and general physical examination (if applicable). The conduct to be followed in the event of each adverse event is detailed in section 9.2 of this protocol.

Additionally, as part of the surveillance of adverse events, each individual will have an "Outpatient Adverse Events Card" (**Annex 9**) where they can record any sign or symptom that occurs outside the clinical site, which will be shown to the clinical investigator for their assessment in the consultations that will be planned to complete the vaccination schedule, or in any other planned contact with the research team (example, evaluation of the immune response on day 56). This form is not obligatory for participants to fill out.

The evaluation of immunogenicity (defined in the protocol) will be done in a subgroup of subjects, **at the beginning** (before the first dose) **and at 56 ± 5 days**: a) seroconversion of anti-RBD IgG antibodies to SARS-CoV-2 at the Immunoassay Center, Havana; b) response of inhibition of the interaction of RBD with its receptor ACE2 in the CIGB, Havana and c) viral neutralization in civil defense laboratories (LISIDA, Havana). The CIGB will freeze biological samples from all the subjects participating in the study for the evaluation of total antibodies and their future use in further research (of the project itself), for the benefit of society.

5.2. Description of the randomization method

A random list will be made for each clinical site. The procedure will be carried out by the Supply Group of the CIGB Clinical Research Direction. To ensure the equiprobable allocation of study groups at (1:1 ratio), a randomized list will be made in blocks of 4 individuals through a computer tool ("2N", developed at the University of Arkansas for Medical Sciences) where each inclusion number will be assigned a study group (CIGB-66 or placebo). This ensures a balance between the groups in the number of individuals included (in each block there will be two subjects who will receive the CIGB-66 vaccine candidate and two others who will receive placebo). The subject's inclusion number will be consecutive.

The study foresees the stratification of subjects in two age groups: 19-50 years old and 51-80 years old where, according to the population behavior of the municipalities where the research will be carried out, about 56% of the individuals are expected to be included in the youngest stratum and 44% in those over 50 years old. To complete it, in each clinical site, after the subject is approved for the research and noted in the "Record of Included and Non-Included Subjects" (**Annex 10**), their identification / location data will be collected in one of the two records of the researcher generated for the trial (**Annex 11**), segregated according to the age group to which the subject belongs (19-50 or 51-80 years). In this manner, at the end of each inclusion day, the number and percentage of subjects included in each record (according to age) will be known and if necessary, the Steering and Organization Committee of the trial will be able to intentionally and opportunely influence the inclusion of a greater or lesser number of subjects in each age group.

Based on the total population and age distribution of each clinical site participating in the ABDALA trial, a number of individuals to be included in each age group will be proposed to each clinical site. This distribution of subjects according to population and age groups may be modified by the sponsoring center (CIGB) during the course of the study, according to the inclusion rhythm that the different clinical sites print, the population that responds to the call for the trial and the quality of the work corroborated during monitoring visits.

At the clinical site there will be a stock with the masked investigational product (see section 7.5), with the medical supplies for its preparation/administration, in sufficient quantities to guarantee the progress of the protocol without interruptions for this concept, being the continuous supply of the vaccines according to the inclusion rate a responsibility of the CIGB.

At the time of inclusion (after verifying the selection criteria and obtaining the subject's consent), the clinical investigator will collect the subject's general information and assign the inclusion number (consecutive, according to a randomized list in his/her possession, starting with the 1st subject). Given the characteristics of the study, the

team of CIGB and MINSAP monitors will have an active presence at the clinical sites that will carry out the research, in order to guarantee the necessary logistics, adherence to the protocol by the clinical investigators and compliance with GCP.

The identity information of the study group to which each subject was randomly assigned (CIGB-66 or placebo) will not know by any of the researchers or volunteers participating in the trial. The randomized lists will be kept under lock by the Supply Group of the Clinical Research Direction of the CIGB.

5.3. Masking

Both the CIGB-66 vaccine candidate and placebo have similar organoleptic characteristics, with no differences between them, which ensured the execution of the double-blind clinical trial.

5.4. Access to the code of the trial participants

In an emergency (for example, a serious adverse event that warrants disclosure of the code), the responsible investigator at the clinical site will contact the main study monitor who will be in charge of opening the code for that particular subject, according to established procedures.

5.5. Identification of subjects

Each subject will be identified by a code indicating the clinical site running the study followed by the "**inclusion number**" (always starting with number **0001**). This identification shall appear in the documents beloning to each individual.

Example, the identification code of the 1st subject included in the "José Martí" Polyclinic of Santiago de Cuba will be: **JM-0001**. In turn, the 1st individual of the "Ramón López Peña" Polyclinic will bear the code: **RL-0001**.

See the "Instructions for filling out the Case Report File" for the identification code of each clinical site in the Abdala study.

5.6. Factors that can be introduced to reduce bias

The following actions, procedures or instructions will be established to eliminate or minimize errors and biases that impede GCP compliance and adherence of individuals to the protocol:

- ✓ Prior to the preparation of this protocol, a meeting was held with clinical specialists and opinion leaders involved in the project, where the strategies for the clinical development of the product were outlined, with the definition of the present experimental design.
- ✓ Once the national drug regulatory agency (CECMED) approves the trial, an inception workshop will be held for analysis, discussion and mastery of the protocol, for the sake of adherence and compliance with GCP by all researchers.
- ✓ The study will be randomized in blocks to ensure homogeneity in the number of subjects included in each group. Likewise, the test will be double-blind and placebo-controlled, methodological pillars in clinical research that provide scientific rigor and credibility.
- ✓ The principal clinical investigator and the monitors of the trial will ensure that the investigational product administered is properly labeled and identified (see Section 7.5) and corresponds to the one assigned in the randomized list. In addition, they will verify that biological samples (serum) intended for immunological evaluations are properly labeled.
- ✓ The investigational product (CIGB-66 or placebo vials) will be provided by the Supply Group of the Clinical Research Direction of CIGB, following their masking procedures and techniques.
- ✓ Given the experience gained by the researchers at the main clinical site (“Saturnino Lora” Provincial Hospital of Santiago de Cuba) during the ABDALA clinical study (phase I-II), some physicians may be designated responsible investigators at different clinical sites participating in this trial, in order to strengthen medical teams. This will be done without weakening the research team of the aforementioned Hospital, which will be responsible for the care of the population belonging to two neighboring clinical sites (Polyclinics “Julian Grimau” and “Armando Garcia”).
- ✓ Quality monitoring visits will be made at all test execution stages, ensuring strict compliance with the provisions of the protocol.

VI. SELECTION OF SUBJECTS

6.1. Study universe

Constituted by those adult subjects, of any sex, permanent residents in the capital cities of Santiago de Cuba, Guantánamo and Granma (with full constitutional rights), apparently healthy or with controlled chronic diseases, who respond to the call for the trial and agree to participate voluntarily.

6.2. Eligibility criteria

1) Individuals with ages between 19 and 80 years.
2) Physical examination without significant alterations (e.g. skin lesions that interfere with local safety assessment, clinically relevant findings).
3) Voluntariness of the subject by signing informed consent.

6.3. Exclusion criteria

1) Subjects with history of infection to SARS-CoV-2 confirmed by PCR-RT or with this virological diagnosis at the time of inclusion in the study.
2) Convalescent, contact or suspected COVID-19 at the time of inclusion.
3) Subjects who have received a vaccine candidate against COVID-19.
4) Acute infection within the last 15 days.
5) Descompensated chronic diseases.
6) Body Mass Index ≤18 or ≥ 35 Kg/m2.
7) Subjects with tattoos in both deltoid regions.
8) Administration of any investigational product in the last three months.
9) Subjects treated in the last three months or with any medical condition that requires during the study some immunomodulator (interferon, transfer factor, biomodulin T, thymosin, etc.), systemic steroid or cytostatic.
10) Have received blood, immunoglobulins, or blood products within three months before the start of the study.
11) Known hypersensitivity to thiomersal and any of the components of the formulation under study.
12) History or suspicion of alcoholism or drug dependence.
13) Pregnancy, lactation or a woman of reproductive age who does not use contraception or is planning a pregnancy.
14) Mental disability to issue consent.

6.4. Criteria for stopping the trial

- ✓ Severe inadmissible toxicity greater than 5% with high probability (> 0.90) and proven causality (Likely or definitive) attributable to the experimental group, stops the clinical trial.

6.5. Withdrawal criteria

All subjects, once they have been included in the study and regardless of whether they subsequently discontinue the study, will be part of the planned statistical analyses, as appropriate. In the case of voluntary dropout, efforts will be made to obtain the cause of the abandonment and all available information.

VII. TREATMENT

7.1. Products that will be used

During the clinical trial, the anti-COVID-19 vaccine candidate will be used: CIGB-66 (PN 5317C), which is a formulation for intramuscular administration. Placebo wil also be used as a formulation for parenteral use. These products must be stored between 2-8 °C.

- **CIGB-66 (Abdala vaccine)** (Composition per each mL - PN 5317C).

Lots (Expiration): RPQ021011/0 (05/2021), RPQ021021/0 (05/2021), RPQ021031/0 (06/2021), RPQ021041/0 (06/2021), RPQ021051/0 (06/2021), RPQ021071/0 (06/2021), RPQ021081/0 (06/2021).

Composition		
Components	**Quantity per mL**	**Part No.**
RBD API	100 µg	4502
Aluminum hydroxide gel (Al^{3+})	0.60 mg	620
Disodium hydrogen phosphate (Na_2HPO_4)	0.56 mg	008
Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$)	0.62 mg	119
Sodium Chloride (NaCl)	8.5 mg	023
Thiomersal	0.05 mg	292
Water for injection	q.s.f.	185

Product characterization and control

The vaccine candidate against the virus SARS-CoV-2 intramuscular CIGB-66 is packaged in 2R vials for injection, DIN standard hydrolytic quality Type I, light crystalline with 13 mm grey chlorobutyl stopper and flip-cap seal.

Quality controls

- Organoleptic characteristics (Visual method): Slightly opaque greyish-white suspension that separates after a sedimentation time in two phases: a transparent liquid and a gel-shaped one that, when stirred, resuspends easily and is foreign particle-free.
- Sterility (SOP 4.09.274.941, SOP 01.5803; EP/USP): Passes the test.
- Pyrogens (SOP 07.0023, USP, Dose in rabbit: 50 µg/ kg animal weight): Conforms.
- Immunogenicity (SOP 4.09.642.201): ≥ 50 % seroconversion
- Percent adsorption of RBD: Micro-Comassie Method; SOP 1.34.604.032): ≥ 50 %

- Identity: (Western Blot, SOP 4.09.470.151; SOP 1.34.602.032): Majority band identification
- Aluminum Ion: (Complexometry; SOP 4.09.064.921; SOP 01.3993): From 0.30 to 0.85 mg/mL
- Thiomersal: (Spectrophotometry; SOP 01.3993): 0.030 to 0.100 mg/mL.
- Volume: (SOP 4.09.271.021; SOP 01.3993; USP): Not less than the volume declared in the label.
- pH: (SOP 4.09.068.921; SOP 01.3993; USP): From 6.00 to 7.00.

➢ **Placebo for intramuscular administration** (Composition per each mL - PN 5319C).

Lots (Expiration): RPQ21011 (06/2021); RPQ21021 (06/2021); RPQ21031 (07/2021).

Composition		
Components	**Quantity per mL**	**Part No.**
Aluminum hydroxide gel (Al^{3+})	0.60 mg	620
Disodium hydrogen phosphate (Na_2HPO_4)	0.56 mg	008
Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$)	0.62 mg	119
Sodium Chloride (NaCl)	8.5 mg	023
Thiomersal	0.05 mg	292
Water for injection	q.s.f.	185

7.2. Route of administration, dose and duration of vaccination

The investigational product (CIGB-66 [50 µg] or placebo) will be administered intramuscularly: 0.5 mL in the deltoid region, every 14 days (short schedule: 0-14-28).

Immunizations will be completed at the clinical site (by the nursing staff responsible for the activity) although the subjects will be on an outpatient basis.

For the administration of the product follow these instructions:

1. For the administration of the investigational product, at each dose: a 2R vial (CIGB-66 or placebo, solution for intramuscular injection) + a 1 mL syringe with 23G x 25 mm needle.
2. Intramuscular injection administrations of CIGB-66 or placebo will be made in the external side of the upper arm (preferably the non-dominant arm of the subject), in the deltoid region, according to the intramuscular injection procedure.
3. For each dose to be applied, it is recommended to use a 1 mL syringe labeled with 10 divisions.
4. Hygienic-sanitary and biosafety measures will be maximized during intramuscular inoculation of the product (CIGB-66 or placebo) and for the disposal of the used material.

Important:

- When the intramuscular application is complete, discard the vial, needle, and syringe. They are placed in the cases in which they were dispensed and returned to the Pharmacy for subsequent collection by the sponsoring center and destruction according to the procedures established in the CIGB.
- Safety standards must always be followed, including the use of gloves, mask and eye protection, to avoid possible transmission of infections. Wash the hands properly.
- If any product volume remains, it will be discarded. All biosafety precautions must be used for waste disposal.

7.3. Rationale for the dose used

The selection of the dose (0.5 mL) strength (RBD 50 μg) and the immunization schedule of CIGB-66 (0-14-28) are based on the clinical results of the exploratory phase 1-2 study, in addition to specialized reports with candidates also based on RBD protein subunits. Preclinical results with this protein were also considered.

As for the RBD, there also are precedents in the clinical trials performed this year to face the COVID-19 in which the concentrations of RBD range from 9 μg to 100 μg.

Pharmacological studies performed, comparing RBD strengths of 25 and 50 μg, evidence of a better response to dose increases (Pharmacological study report CICUAL/CIGB/20074).

Vaccine candidates in development and dozens already in clinical trials include the RBD region. Of these candidates, more than 70 are of subunits and the following lists that include only RBD, are already in different phases of clinical studies[54].

- BioNtech Anhui Zhifei Longcom Biopharmaceutical (Subunit)
- West China Hospital, Sichuan University Fase I (Subunit)
- Anhui Zhifei Longcom Biopharmaceutical (Subunit)
- Clover Biopharmaceuticals (Subunit)
- Kentucky Bioprocessing (Subunit)
- Adimmune (Subunit)
- Instituto Finlay de Vacunas (Subunit)
- SpyBiotech (Subunit)

Of these, BioNtech's BNT 162b2 is an mRNA vaccine coding only for the RBD region, began phase I trial in the US with 360 volunteers and is in phase III (43,000 subjects in the US, Argentina, Brazil and Germany). They evaluate doses of 60-100 μg of the vaccine candidate. On the other hand, Clover Biopharmaceuticals with a candidate

expressed in CHO studies in phase I doses up to 30 μg of antigen. The candidate of Anhui Zhifei Longcom Biopharmaceutical consists of RBD expressed in CHO, as well as that of the Finlay Institute of Vaccines, Havana, both with doses up to 50 μg antigen in a schedule of three administrations.

When evaluating the results of the exploratory clinical study (Abdala, phase I-II) carried out at the "Saturnino Lora" Provincial Hospital in Santiago de Cuba, the safety and immunogenicity results were corroborated, which support the continuity towards a phase III efficacy study, using CIGB-66 at a dose of 0.5 mL, strength RBD 50 μg in the short 0-14-28 schedule.

In terms of safety the CIGB-66 vaccine candidate is safe in the strengths (RBD 25 and 50 μg) and studied schedules (0-14-28 and 0-28-56). As for immunogenicity, for the short schedule, in the analysis of seroconversion of anti-RBD IgG antibodies of the SARS-CoV-2 (which considered seroconversion as that ≥ 4 times the initial determination of antibody titer) seroconvert more than 64% at 42 days in subjects who received the least strength (25 μg), while for the group of 50 μg it is stated with 95% reliability that more than 72% seroconverts on day 42 and 85% on day 56; and the probability that the immune response will be achieved in more than 50% of individuals is 0.99 since day 42. Also, for the group of 25 μg the geometric mean of anti-RBD IgG-specific antibody titers can be found with 95% reliability higher than 13.1 and less than 38 also since day 42. On the other hand, in the 50 μg group the geometric mean of anti-RBD IgG-specific antibody titers can be found with 95% reliability greater than 71 and less than 244 on day 42; which increase slightly at day 56, where the lower limit is 83 and the upper limit is 291. In terms of percentage of inhibition it is observed, with 95% confidence that it is greater than 24% on day 42 and 25% on day 56 in the 25 μg group; while it is greater than 52% and 64% for the same respective times in the 50 μg group. The mean of the percentage of inhibition in the 50 μg group is higher than the 25 μg group; confidence intervals are exclusive, (29.8, 55.8) and (55.6, 81.3) in respective groups of 25 μg and 50 μg. And when calculating the percentage of subjects with seroconversion index ≥4 and who inhibited more than 30%, in the 25 μg group were 10/21 subjects (47.6%) and in the 50 μg group were 19/21 subjects (90.5%).

Taking into account the dose-response relationship observed in pharmacological studies and clinical studies of COVID vaccines under execution in the world, which evidences the need to increase the dose of the antigens when passing to the evaluation in humans and also considering the safety references of the above mentioned vaccines, and the results of the Phase 1-2 study executed in Cuba with the vaccine candidate CIGB-66, the maximum strength evaluated (50 μg of RBD antigen) was selected for the continuity of the clinical development of the Abdala study (Phase 3).

The distance between the administrations of vaccine doses facilitates the maturation of the immune response, resulting in the selection of antibody-secreting clones of increased avidity and memory response. Nontheless, short immunization schedules could also be of practical use in promoting a faster protective response, necessary for outbreak control and protection of personnel at immediate risk, such as travellers to pathogen circulation areas. Increased maturation and functionality of the immune response also contribute to the administration of multiple doses.

In the example of hepatitis B vaccines, WHO recommends a variety of schedules (0-1-6 months), (0-1-2-12 months) and also accepted by regulatory authorities (0-7-21 days)[13]. For this vaccine, although a short immunization schedule is lower in terms of ensuring long-term protection, but maintains its usefulness where rapid interventions are required to achieve protection of subjects.

With all these elements, although in the future the long schedule (0-28-56) could be considered in different intervention strategies, or in clinical trials in other age groups and contexts, the country, like the world, is currently experiencing an epidemiological situation that requires fast, effective and safe interventions. The use of a short schedule, such as that proposed in this research, could respond to this necessary immediacy, with a benefit-risk ratio broadly inclined towards benefit.

7.4. Rules for the use of concomitant treatments

The volunteers will know that, for the duration of the clinical trial, they should not receive other vaccines, experimental products, make blood donations, and administer immunosuppressants or immunomodulators in general, unless they are strictly necessary for their health.

The subjects will be questioned about the habitual use of medications, as well as basic treatments, the details of which must be recorded in the model related to concomitant therapy generated for the purposes of the study. Likewise, should the need for concomitant medication arise, this will be analyzed and ultimately indicated by the physician, and the details will be recorded in the said model.

Any other treatment that the volunteer receives outside of the clinical site setting should be reported and recorded by the physician. The investigator should weigh whether the medical condition occurred and/or medical intervention required, should be considered for discontinuation of vaccination. It should also be considered if it is an adverse event. If any allergic reaction occurs, antihistamines and steroids will be used for treatment, as the case may be (at the discretion of the physician). The behavior before any adverse event will be decided by the physician depending on the type, magnitude and severity of the clinical manifestations in each case.

7.5. Measures to guarantee safety in product handling

The preparation and administration of the product will be carried out by personnel trained for these purposes, under the indication and supervision of the investigator. The product (CIGB-66 or placebo) will be stored from 2-8ºC (Do not freeze) either at the sponsoring center, during transportation, or while stored at the research site or another Public Health Unit or BioCubaFarma.

There will be a "Vaccination Record", unique for each clinical site, where strict control of compliance with the immunization schedule of all subjects included in each clinical site will be centrally controlled (**Annex 12**). Each time the subject receives the corresponding dose of the research product, the date and time (and the nurse's signature) will be recorded. Depending on the facilities created at each clinical site to comply with the flowchart foreseen in the study, as well as the number of subjects to be attended each day, the work has been organized in such a way that individuals come to the clinical site in a staggered manner, between 08:00 - 18:00 hours each day. Likewise, to guarantee an organized work flow, **≥ 2 vaccination stations will be established in most clinical sites** (each station with two nurses: one vaccinating and the other registering the vaccination act in the corresponding record and providing the vial of the investigational product, coded, randomly assigned to the subject in question). **All these stations or vaccination points, in each clinical site, should be in the same room** (spacious, ventilated, with an organization that allows the work to be carried out separately for each station), since the vaccination record is unique and must be accessible to all those who will perform the activity; otherwise, the work and concentration of the nurses in the most important activity of the whole process will be hindered, especially in the case of a double-blind study, where a vaccine candidate and a placebo are assessed). Moreover, each clinical site has only one refrigerator for this activity (at the vaccination station) and a pharmacist in charge of the supply activity.

On the other hand, in order to guarantee the necessary rest for the nurses responsible for the vaccination and so that the number of subjects to be vaccinated per day is not excessive, there will be a rotation (at each vaccination station) of the most qualified nursing personnel who perform other functions within the Abdala study itself (vital signs station, observation room for vaccinated subjects, facilitators, etc.). Another alternative could be to increase the number of nurses dedicated exclusively to the vaccination activity (organized by brigades, with work-rest schedules, always guaranteeing the activity in the number of subjects to be vaccinated each day).

CIGB-66 or placebo vials will be shipped to each clinical site, in cases, segregated by appropriately coded study groups. Clinical sites should ensure the necessary supplies for the preparation/administration of the investigational product (1 mL disposable syringes, 23G x 25 mm needles, alcohol, swabs, etc.).

In the days foreseen in the trial schedule for the immunization of subjects, the CIGB shall be responsible for organizing the transfer to the clinical site of the investigational product. Taking into account the considerable volume of vaccines that will be applied at each clinical site participating in the trial, as well as the limited storage capacity of these care units, CIGB has coordinated with another company of BioCubaFarma(EMCOMED Santiago, Guantanamo and Granma) the storage of the investigational product in certified cold chambers that guarantee adequate cooling. The CIGB (through the Supply Group of the Clinical Research Direction) will be in charge of indicating the timely and gradual supply of this product at each site.

At all times the cold chain will be guaranteed (2-8ºC). The refrigerator where the investigational product is kept will keep a temperature record that will be checked at least twice a day. These temperature conditions will also be taken into account during the transportation of the product, which will be the responsibility of the CIGB.

At the end of the study, the sponsoring center will collect the empty (and not dispensed, if applicable) vials, which will be destroyed in the CIGB according to the planned procedures.

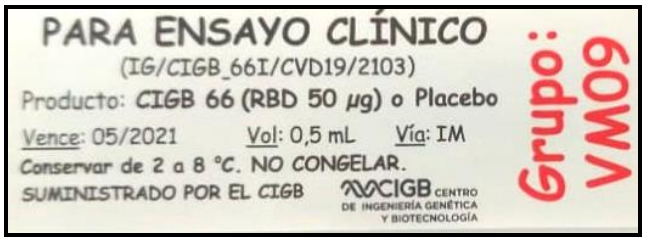
As it is a double-blind study, there are rules to be complied with to ensure the proper masking of the investigational product. In this regard, the label on the case will specify the identification code of the study group (e.g. **CC08, VM09, JL10 or FH12**), the name of the two investigational products (CIGB-66 or placebo), the expiration date (expiration date of the investigational product that first expires), the route of administration (intramuscular), the storage temperature (2-8ºC; Do not freeze), and the phrases “For clinical trial” and “Supplied by CIGB”, the short name of the study and its code (ABDALA, IG/CIGB-66I/CVD19/2002), as well as the name of the hospital. The lot is not included so as not to disclose the product.

Example of case label text:

The vial label (CIGB-66 or placebo) shall also specify the study group identification code (e.g. **CC08, VM09, JL10 or FH12**), the name of the two investigational products (CIGB-66 or placebo), the expiration date (expiration date of the investigational product that first expires), the route of administration (intramuscular), the storage temperature

(2-8ºC; Do not freeze), and the phrases “For clinical trial” and “Supplied by CIGB”.

Example of CIGB-66 or placebo case label text:



These products must be stored in their original case (supplied by CIGB), in a refrigerator intended for this purpose. The Head of the Pharmacy (or the specialist designated in the clinical site) will be responsible for the reception, storage, conservation and dispensing of the investigational product destined for the clinical trial; ther must be a control of the entrances and exits of the investigational product. During quality monitoring visits, dispensing records, dispensed product (empty vials) and temperature control records will be checked.

In the event of accidental damage to a vial or if foreign particles and/or color change are detected (it must be slightly opaque grayish-white), **the ENTIRE vial is discarded**, the event is recorded, the main monitor is notified and another vial with the same code from the study group assigned to the subject is taken.

7.6 Measures to promote and control that instructions are followed

Measures to ensure compliance with the prescribed instructions are specified below:

- The logistics to be followed at the clinical site to ensure the process of selecting subjects for the test, obtaining informed (written) consent and the timing of administration of the 1st dose of the investigational product (CIGB-66 or placebo according to randomized listing) will be established in a detailed/explicit manner, so that full adherence to GCP and the test protocol is achieved by the research team.
- Prior to the official inclusion in the clinical trial, recruitment of potential study volunteers will be carried out (at the level of the family doctor’s offices selected by each clinical site). These family doctors, supported by other physicians at the clinical site, will make the necessary talks and calls as part of the survey of all potential candidates for the study. Those who come to the doctor’s office will be asked for informed consent (after they have read the information sheet for the subjects that will be given to them, and have the time they deem appropriate to make their decision) and a pre-inclusion check will be made, where the eligibility criteria for the study will be verified. The result of this check-up is collected in the “Case Report File” - CRF (**see annex to the protocol**), which will be supplied to the individual (together with the consent signed and dated by the subject

and the physician) to be delivered to the Abdala - Phase 3 inclusion consultation, at the clinical site according to the established schedule.

- Each subject will be evaluated throughout the study by the same medical team that included him/her, who will be in charge of filling in the primary information generated for the purposes of the clinical trial. Personnel not linked to the study will not be allowed to attend the cases or access the investigational product.
- The deliveries of the product to the clinical site, duly identified, will be guaranteed by the sponsoring center, which will be recorded in a distribution control form.
- The use of the investigational product will be reserved only for the subjects participating in the trial. All clinical researchers will have access to the product, which will be duly stored at the clinical site. Given the characteristics of the trial, where a significant number of volunteers will be immunized in the same day (as organized), the Supply Group of the Clinical Research Direction of CIGB will establish the requirements for the mass extraction of the investigational product from the cold chambers of EMCOMED.
- Compliance with vaccination shall be strictly monitored by means of a record allowing the traceability of the activity (**Annex 12**).
- Quality certificates for investigational products (CIGB-66 and placebo) will be provided to the clinical site prior to use. It should be retained in the Pharmacist's Folder.
- Researchers will fill out a record of subjects included (including number) and non-included (indicating cause). This form should only show the subject's initials as a contribution to data confidentiality (**Annex 7**). This form will be sent to the CIGB at the end of the clinical trial.
- The responsible researcher at each clinical site will have an internal record of the subjects included where their personal data can be shown in full. This form will allow individuals to be located in any situation, including planned absences for consultations (**Annex 8**). It will be retained at the clinical site and no copy will be generated for the center sponsoring the study.
- The clinical researchers will participate in a Criteria Unification Workshop with the aim of achieving understanding/adherence to the approved protocol, and will be trained in GCP.
- The laboratory determinations foreseen in the protocol for the evaluation of the immune response will be performed by CIGB personnel with experience in the techniques and using standardized methodologies.
- Empty vials after use and those that are not used shall be returned to the sponsoring center for destruction in accordance with the procedures laid down in the CIGB.

- During the progress of the research, quality monitoring visits will be carried out by the CIGB monitors, where the observance of the provisions of the protocol, fidelity in data collection, safety profile (adverse events), will be verified, as well as compliance with the GCP. In addition, the clinical site will be subject to state inspections when decided by the national drug regulatory agency (*Center for State Quality Control of Drugs and Medical Devices - CECMED*).

The principal clinical investigator will be responsible for compliance with the foregoing.

7.7. Causes of vaccination discontinuation

The causes of discontinuation and which may affect the investigational product administration stage are:

1) Patient dropout.
2) Death of subject.
3) Serious adverse event (in relation to proven causality attributable to the investigational product).
4) Hypersensitivity reaction to product administration.
5) Repeated adverse events with moderate intensity intolerable to the individual.
6) Appearance of any exclusion criteria detected after inclusion.

Note: These moderate adverse events could result in definitive discontinuation if the same event was repeated in the next administration of the investigational product unless, exceptionally, the physician decides to maintain administrations when considering the risk-benefit balance, and the consent of the individual.

Voluntary dropouts due to “loss of follow-up” will be considered the subjects who were repeatedly absent from the visits scheduled in the trial and with which the clinical site fails to establish contact.

VIII. EVALUATION VARIABLES

8.1. Variables to measure response

8.1.1. Primary endpoint

- **<u>Vaccine efficacy</u>: Number of symptomatic COVID-19 subjects without evidence of prior exposure to viral infection**

<u>Case definition</u>: For efficacy, patients with RT-PCR positive to SARS-CoV-2 will be used, **from 14 days after the 3rd dose of the investigational product** and who present:

- At least one symptom or major sign **or**
- At least two of the symptoms or minor signs of COVID-19.

<u>MAJOR symptoms and signs</u>:

- New onset dyspnea or worsening.
- Saturation of $O_2 \leq 92\%$ by pulse oximetry without oxygen supplement.
- Persistence of chest pain.
- Change of behavior or states of alteration in the state of consciousness.
- Local or generalized cyanosis.
- Pneumonia by clinical or imaging diagnosis.

<u>MINOR symptoms and signs</u>:

- Fever (≥38 ˚C) or febricula (37.1 – 37.9 ˚C).
- New onset cephalea.
- Chills.
- Odynophagia.
- Congestion and/or runny nose (only one finding will be counted for the definition of the outcome variable).
- New onset cough or worsening.
- Myalgias.
- Fatigue that interferes with daily activity.
- Vomiting and/or diarrhea (only one finding will be counted for the definition of the outcome variable).
- Anosmia/ageusia (only one finding will be counted for the definition of the outcome variable).

Once the subject is SARS-Co-V-2-positive by PCR, detected by the Provincial Center for Hygiene, Epidemiology and Microbiology (CPHEM) of the provinces involved, it will be detected by several routes:

- ✓ The results of these individuals are reported, according to the national action protocol against COVID-19, to the clinical sites where the subjects belong, which are the same Polyclinics participating in the ABDALA - phase III clinical study, resulting in an alert for the research team that has the record of participating subjects.
- ✓ Also, together with the company GEOCUBA, CIGB designed a computer tool (APK), which will be used in each clinical site as part of the control of immunization compliance. It will scan the individual's ID card each time the investigational product is administered. With the APK, a positive PCR case can be detected as soon as the subject is reported by the CPHEM (thanks to the identity number).
- ✓ Finally, all subjects participating in the clinical trial will have an Identity Card (**Annex 13**) where they will be instructed to show it to the attending physicians so that they know that they are participating in this research and that they should contact people responsible for the ABDALA study, to proceed immediately with the transfer to the "Joaquín Castillo Duany" Military Hospital in Santiago de Cuba (care unit for the centralized care of all PCR-positive cases of all clinical sites participating in the study).

8.1.2. Secondary endpoints

1) Prevention of mild, moderate and serious forms of COVID-19 following the same criteria that are internationally defined when addressing the clinical spectrum of SARS-CoV-2 infection[55], which are listed below:

- **Mild disease:** Individuals who have any of the various signs and symptoms of COVID-19 (example: fever, cough, odynophagia, malaise, cephalea, myalgia, nausea, vomiting, diarrhea, loss of taste and smell), but who do not have difficulty in breathing, dyspnea or abnormal chest images.
- **Moderate disease:** Individuals showing evidence of lower respiratory disease during clinical or imaging evaluation, with oxygen saturation (SpO_2) ≥94%.
- **Serious disease:** Individuals who have SpO_2 <94%, a ratio of oxygen partial blood pressure to inspired oxygen fraction (PaO_2 / FiO_2) <300 mmHg, a respiratory rate >30 breaths/min, or pulmonary infiltrates >50%.
- **Critical disease:** Individuals with respiratory failure, septic shock and/or multi-organ dysfunction.

2) Safety. Clinical adverse events: The type, duration, intensity, cause-effect relationship, followed behavior and outcome will be described (See paragraph IX of adverse events). To do this:

- ✓ The subject will be evaluated in each dose through anamnesis, physical examination, vital signs and inspection at the inoculation site: **pior** to the administration of the product, **in the first hour following the inoculation** of the product (**CRF, Annexes 6 and 7**). Observations at the clinical site include anamnesis, vital signs (temperature, blood pressure, respiratory and cardiac frequencies), inspection of the inoculation site (for the detection of local symptoms), and general physical examination.
- ✓ Subsequently, with the support of medical students (in higher years) and family physicians in the Community, 20-30% of the vaccinated individuals will be visited each day (within 72 hours after each dose) with the intention of identifying and characterizing the adverse events that may occur (**Annex 8** "*Active screening for adverse events in the field*").
- ✓ On the other hand, subjects will be instructed on the need to contact the ABDALA study research team in case of any medical eventuality. This monitoring will be carried out in the period between each dose (14 days). Additionally, as part of the surveillance of adverse events, each individual will have an "Outpatient Adverse Events Card" (**Annex 9**) where they can record any sign or symptom that occurs outside the clinical site, which will be shown to the clinical investigator for their assessment in the consultations that will be planned to complete the vaccination schedule, or in any other planned contact with the research team (example, evaluation of the immune response on day 56). This form is not obligatory for participants to fill out.
- ✓ Similarly, the subjects will be monitored throughout the duration of the test, and even in the long term outside the protocol (minimum up to one year) by their respective Health Areas (family doctors) where any medical incident of interest to the project will be reported (for example, if a pregnancy/delivery went normally, diseases, etc.).

The conduct to be followed in the event of each adverse event is detailed in section 9.2 of this protocol.

3) Proportion of subjects with anti-RBD IgG antibody seroconversion of SARS-CoV-2 (in a subgroup of subjects). Seroconversion will be considered as that ≥ 4 times the initial determination of the antibody titer, at 56 ± 5 days compared to baseline time.
4) Geometric mean of anti-RBD IgG-specific antibody titers, on days 0 and 56 ± 5.
5) Inhibition of interaction of RBD with its ACE2 receptor by ELISA, on days 0 and 56 ± 5.
6) Percentage of viral neutralization response to ARS-CoV-2 on days 0 and 56±5. In this case, there will only be evaluated those samples that have ≥ 50 % of inhibition at a 1/100 dilution in the neutralization test of the RBD

binding to the ACE-2.

The following table summarizes the immunological determinations to be performed:

Evaluation	Frequency	Type/quantity of sample	Extraction volume	Analytical technique	Analyzing laboratory
Secondary endpoints					
Seroconversion of anti-RBD IgG-specific antibodies (that ≥ 4 times the initial antibody titer determination).	**0** and **56 ± 5 days**	Serum / 2 mL	5 mL	ELISA	Analytical Laboratories of the CIE and CIGB, Havana
Geometric Mean of the anti-RBD IgG-specific antibody titers				ELISA	
Percentage inhibition of the interaction of RBD with its ACE2 receptor.				ELISA	
SARS-CoV-2 Viral Neutralization Response Only those with ≥ 50% inhibition at 1/100 dilution in the ACE-2 RBD binding neutralization test will be assessed.				Viral neutralization	Civil Defense Laboratory

In addition, the cell response will be evaluated as a screening variable:

Evaluation	Frequency	Type/quantity of sample	Extraction volume	Analytical technique	Analyzing laboratory
Cell response (IFN γ)	0 - 70	Whole blood	CPT 9 mL tube	ELISPOT	Analytical Laboratory, CIGB

In order to determine the serological status of the individuals included in the test and determine whether they had previous contact with the SARS-CoV-2 virus, 100% of the subjects included in the study will be tested for total anti-SARS-CoV-2 antibodies in the zero-time sample.

This evaluation will be performed at the Immunoassay Center, using the UMELISA ANTI SARS-CoV-2, which allows the detection of total antibodies in human serum or plasma samples. This study is relevant for understanding the extent of COVID-19 in a given community, as well as identifying immune individuals who are therefore potentially "protected" from future infections. This aspect is relevant for the evaluation of the efficacy of vaccine candidates, which must be determined in all individuals who are no longer in that condition.

In the laboratories of the CIGB in Havana, the Immunoassay Center (CIE) and Civil Defense, the immunological determinations (in serum) defined in the protocol will be made. Although they will be performed in a subgroup of subjects, the protocol foresees the collection of these biological samples in all participants, **at baseline** (before the first dose) **and at 56 ± 5 days**, as specified in these secondary endpoints. The CIGB will keep these samples

frozen, with the possibility that (if the conditions are created) the immune response can be evaluated in all individuals or they can be used in the future in further research (of the project itself), for the benefit of society.

Biological samples (serum) will be obtained under optimal biosafety conditions at the clinical site and will be frozen at -20°C until they are transferred to the CIGB where the planned immunological evaluations will be made.
The sponsoring center will be responsible for the operational transfer of the procedures for the extraction, identification and conservation of the biological samples that will be transferred to the CIGB to fulfill the objectives foreseen, according to procedures 4.40.121.01 and 4.40.122.01 in force at the Clinical Research Direction of the CIGB. In the same way, all participating personnel must pay special attention to the handling of the samples, as they are considered capable of transmitting infectious agents.

The transfer of the samples from the clinical site will be the responsibility of the sponsoring center (CIGB), which will guarantee the transportation, specialized personnel and resources necessary to perform these operations with the highest quality and compliance of GCP, as established by the current procedures (4.40.120.01 and 4.40.123.07) and biosafety protocols.

Biological samples destined to the CIGB:

The process of obtaining serum from the blood samples will be performed in the clinical laboratory of the own health center that will conduct the study, according to the routine procedures established for blood chemistry determinations. The serum aliquots destined for the CIGB will be stored, suitably at -20°C (or frozen in a domestic refrigerator), until they are collected and transferred to the center sponsoring the study.

Sample processing: *To obtain SERUM* *(a suggested procedure, although there are other methods):*

- Incubate blood samples (anticoagulant-free) intended for obtaining serum at 37 °C for one hour.
- Then, incubate at 4°C for 30 minutes.
- Centrifuge at 3000 rpm for 15 minutes.
- Extract the serum without breaking the clot. Transfer to a plastic freezing vial. Identify the vial as appropriate. Discard the clot.
- Store the serum at -20ºC until analysis.

Identification of biological samples (destined for the CIGB / CIE / LISIDA):

- With the intention of keeping the laboratory analysts responsible for the immunological evaluations blind, each biological sample (serum) will be initially identified with a label specifying the short name of the test

(ABDALA Study - Phase III), the type of biological material (**serum**) and the identification code of the subject followed by the extraction time (Example: **JM-0001-T0** or **JM-0001-56d**). Subsequently, the samples will be recoded at the Clinical Research Direction of the CIGB and submitted to the Biomedical Research Direction for immunological evaluations. This recoding (involving label substitution) will carry a five-digit code, which will be unique to the subject and unrepeatable, unrelated to the sample code of that individual at another extraction time. The reason for not proceeding with the latter coding at the clinical site itself is due to the cumbersome nature of the technique (which requires great care and concentration) and the considerable number of biological samples that will have to be processed each day.

The responsible investigator will supervise the activity and the correct filling of the "Biological Samples Record". The lab technicians involved in the test will be responsible for compliance with the activity, adherence to the protocol and proper preservation of the biological samples (until they are delivered to the sponsoring center).

8.1.3. Control variables

- Age (years)
- Skin color (white, mestizo, black, yellow).
- Sex (masculine / feminine).
- Body Mass Index (Kg/m^2).
- Toxic habits (smoking, alcohol consumption).
- Personal pathological history / Risk factors (Yes, No, Type).
- Concomitant treatments to the investigational product.
- Previous SARS-CoV-2 infection.

8.2. Criteria to measure response

Safety criteria

✓ The product will be considered safe and well tolerated in a subject when not associated with adverse events grade ≥ 3 according to CTCAE *(Common Terminology Criteria for Adverse Events, v5.0: November 27, 2017)*[56].

Criteria to assess specific immune response

✓ Anti-RBD IgG antibody seroconversion of SARS-CoV-2 estimated by ELISA (considering seroconversion as ≥ 4 times the initial determination of antibody titer).

- ✓ Geometric Mean of the anti-RBD IgG-specific antibody titers. The titers are logarithmically transformed and averaged, then compared between vaccination groups and at different evaluation times.
- ✓ Ability of serum to block the interaction between the RBD protein and its ACE2 soluble receptor, evaluated by ELISA; for this purpose, the inhibition rate of the binding between RBD and its ACE2 receptor produced in a serum of a vaccinated individual is calculated by a formula.
- ✓ Viral neutralization response SARS-CoV-2 (evaluation in cases that have ≥ 50% inhibition at a 1/100 dilution in the neutralization test of RBD binding to ACE-2).

8.3. Criteria for success or failure of the study

👍 It will be considered **individual success** if the subject is not COVID-19 symptomatic, if no serious adverse events with proven causality occur and if seroconversion of SARS-CoV-2 anti-RBD IgG antibodies is achieved.

👍 **Therapeutic success** will be considered if the hypothesis is demonstrated.

👎 It will be considered **individual failure** if the individual is COVID-19 symptomatic, definitively discontinues vaccination due to serious adverse events attributable to the investigational product or if seroconversion of SARS-CoV-2 anti-RBD IgG antibodies is not achieved.

👎 **Therapeutic failure** is considered if the hypothesis of the study is not demonstrated.

IX. ADVERSE EVENTS

9.1. Adverse events that may appear and methods for recording them

Adverse events will be classified according to type, duration, intensity, causal relationship, conduct followed and outcome, in the following grades[56]:

Grade **1** ***(Mild)***	Asymptomatic or symptoms of mild intensity. Clinical or diagnostic observation only. Do not require treatment.
Grade **2** ***(Moderate)***	It requires minimal, local or non-invasive intervention.
Grade **3** ***(Serious)***	It is not immediately life-threatening. Requires hospitalization (or prolonged hospitalization). Disabling.
Grade **4** ***(Serious)***	Life-threatening. Requires urgent intervention.
Grade **5** ***(Serious)***	Adverse event-related death.

The analysis of the cause-effect relationship between the adverse event and the investigational drug will be carried out using the following qualitative analysis[57]:

1. Definitive: An event that **1)** shows a reasonable time relationship; **2)** follows a known or plausible response to the investigational drug; **3)** there is no reasonable explanation that is produced by other factors such as the patient's condition or concomitant drugs administered; **4)** disappears after administration is discontinued and reappears when the exposure is restarted.
2. Likely: An event that **1)** shows a reasonable time relationship after drug administration; **2)** shows a known response pattern of the investigational drug; **3)** cannot be explained by other factors such as the patient's clinical status or concomitant drugs administered; **4)** disappears after the administration is discontinued but is not confirmed with re-exposure.
3. Possible: An event that **1)** shows a reasonable time relationship; **2)** may or may not follow a known response pattern to the investigational drug; but **3)** it may be produced by other factors such as the patient's clinical status and concomitant drugs administered.
4. Doubtful: The event is more likely related to other factors than to the drug involved.

Although it is a novel product, RBD proteins obtained from different expression models have been evaluated in a large number of volunteers, with an adequate safety profile. Likewise, the adjuvant has been used extensively in humans. The main adverse events that may occur are pain/burning at the injection site, erythema, heat sensation, general malaise, fever, cephalea, among others.

9.2. Behaviour to follow against adverse events

In this study, no serious adverse events are expected to occur with intramuscular administration of the CIGB-66 vaccine candidate that cannot be controlled without major consequences. In this clinical trial (ABDALA - Phase III), in the event of an adverse event, it will be followed progressively and all the information concerning such incident will be collected (description of signs and symptoms, duration, behavior to mitigate it, etc.). The physician should evaluate the cause-effect relationship between each adverse event and the investigational drug (according to the criteria described in section **9.1**). In case of serious adverse events, vaccination will be discontinued, the required measures will be taken depending on the type of event and the IDMC will deepen in the reported event and propose to the Sponsor the conduct to follow.

All subjects, once vaccinated, move to an observation area for one hour, under the permanent supervision of a nurse (with accessibility to a stretcher and/or wheelchair). At the end of the observation, all subjects have their vital signs taken and the physician interviews/examines for local or systemic adverse events. If no event is reported, the subject is removed from the clinical site and instructed on how to proceed in the event of a subsequent medical problem (procedure detailed in section 8.1.2.). In the event of any medical eventuality, depending on its nature, the appropriate measures will be taken:

- ☞ Mild / moderate adverse events (reported by the subject or detected during vital signs or physical examination): depending on the type, intensity, cause-effect relationship with the investigational product, etc., pharmacological / non-pharmacological therapy will be adopted at the discretion of the physician, if appropriate. If an allergic reaction occurs, it can be controlled with the administration of antihistamines and steroids, depending on the case.. At the vaccination station there will be a stock of essential medicines to support a timely medical procedure (including analgesics, steroids, antihistamines, antihypertensives, among others).
- ☞ Serious adverse events: A life support area (with cardiac arrest car, defibrillator, as well as drugs and supplies established to deal with a medical emergency) has been guaranteed at each clinical site. Also, within the medical team involved in the study, there will be qualified clinicians trained in emergency medical care. Likewise, while the study lasts (and in particular, in the vaccination days), the SIUM units will be available for the transfer, with the greatest immediacy, of the subjects to the secondary level of medical attention.
- ☞ In case of a medical event that requires urgent attention, after the subject leaves the clinical site, depending on the time and place, should be immediately transferred to a hospital unit (unless the nature of the adverse event can be dealt with at the clinical site itself). In any case, subjects will be asked to inform the attending physicians at the Hospital that they are volunteers in the Abdala - phase III study, to whom they can show the “identity

card" that will be given to them (**Annex 13**), where telephone numbers appear to locate and report to those responsible for the study any situation of interest for the research.

The clinical investigator will be in charge of the diagnosis and follow-up of subjects with adverse events.

9.3. Expedited reporting of adverse events

When a serious unexpected adverse event occurs, the clinical investigator will notify (within 12 hours) the Clinical Trial Sponsor (CIGB) and the REC / SREC. Information may be communicated by telephone, e-mail or in person. The sponsor shall ensure timely notification to the Cuban health authority (CECMED) within the following deadlines:

1. In the case of a serious and unexpected adverse event that causes death or threatens the life of the subject, it is reported as soon as possible after the occurrence of the reaction becomes known.
2. In the case of a serious and unexpected adverse event that is not fatal or life-threatening, shall be reported as soon as possible and not later than 15 calendar days from the first known occurrence of the reaction.

The Unexpected Serious Adverse Event Report, issued by CECMED in its Regulation 45-2007 (Requirements for Notification and Reporting of Unexpected Serious Adverse Events in Clinical Trials), will be completed at the clinical site. This form (general in studies conducted in Cuba) will be available.

It is the responsibility of the clinical investigator to communicate to the study monitor any serious adverse events that occur and to determine the measures to be taken in each case to protect study participants.

CIGB monitor to contact: PhD. Francisco Hernández Bernal ☎ 72080428 / 72087465 (ext. 148).
Corporate mobile line: 52168101 ✉ hernandez.bernal@cigb.edu.cu

X. DATA COLLECTION AND HANDLING

10.1. Data collection form

Form	Time in which it is filled	Information it collects	Responsible
Consent / Subject information **(Annex 4)**	Before inclusion.	Voluntary confirmation, in writing, of the subject participating in the trial.	Clinical researcher
Consent / Subject information **(Annex 5)**	Before inclusion.	Summarized information about the trial in question, its objectives, potential benefits, rights, risks and inconveniences.	Clinical researcher
Vaccination completion consultation **(Annex 6)**	When the subject receives the 2nd and 3rd doses of the investigational product	Control of attendance at the vaccination program consultation; vital signs (before and after each dose), etc.	Clinical researcher
Medical Record of Adverse Events (**Annex 7**).	When a medical eventuality is reported by the subject.	Recording and characterization of each adverse event reported during the study, in the periods corresponding to each dose of the product.	Clinical researcher
Active screening of adverse events in the field (**Annex 8**)		Active surveillance of adverse events in the community. Recording and characterization of each adverse event reported during the study, in the periods corresponding to each dose of the product.	Medical Students and Family Physicians.
Adverse Events Outpatient Card **(Annex 9)**	At the time the information is generated.	Recording (by the subject participating in the study) of adverse events occurring while on an outpatient basis.	Clinical researcher
Record of included and non-included patients. **(Annex 10)**	Case inclusion period.	List of all subjects who responded to the trial call (included or not and causes).	Clinical researcher
Researcher's Record **(Annex 11)**	When the subject is included in the study.	Identification data of the participants, with general information for quick location.	Clinical researcher
Vaccination Record **(Annex 12)**	When the dose of the investigational product is applied.	Control of vaccination compliance at each dose, in each of the subjects included.	Vaccinating nurse
Record of authorized signatures in the clinical trial **(Annex 14)**	Prior to the start of the study.	Control of authorized investigators' signatures upon completion of the primary information generated in the study (at the clinical site and at the CIGB).	Monitor
Case Report File	During precheck and 1st dose of the product	Demographic and baseline characteristics of the subjects, pathological history, baseline vital signs, verification of the selection criteria for the study, control of the extraction of the baseline biological sample and inoculation of the 1st dose, follow-up at one hour post-vaccination.	Clinical researcher

The informed consent forms will be filled out using the *"not copy required"* system (self-replicative sheet), which allows the immediate generation of copies of the original document. The responsible investigator will retain the original document (for the established period of 15 years, as part of the primary information of the clinical test) and will provide the subject participating in the study with a copy of it (together with the information sheet - **Annex 4**).

The rest of the records and data collection forms of the primary information (where the information source will be generated) will be submitted to the CIGB for data management and statistical analysis, in addition to their proper conservation for the foreseen time (15 years). Given the characteristics of this study, which will require the simultaneous attention of thousands of volunteers, it makes it impossible to have a clinical history. However, there will be documented traceability of all screening, inclusion, medical care, follow-up of adverse events, among others, which will constitute the primary information of this clinical trial. Regarding the evaluation of efficacy in subjects who meet the case definition (symptomatic COVID-19), who will receive centralized care / evaluation at the Military Hospital of Santiago de Cuba, as well as those contacts / suspected individuals included in the Abdala study (who will go to an isolation center), a medical history will be made, according to the working procedures in medical care, where the COVID-19 protocol will be completed for these cases.

The information requested in the different forms and primary records will be completed by the researchers as they are generated. After the last evaluation, where appropriate, the forms are submitted to the CIGB. Before that, the researcher responsible for the study will verify the answers to the questions or information requested and that no section has been left blank. In those questions where it is not possible to obtain the information, proceed as indicated in the "Instructions for filling in the primary information". The annotations will be made preferably in black ink or blue ink, and neither crossing outs nor blotches, illegible letters or words will be produced. If it is necessary to make any corrections, incorrect datum or value shall be crossed out with a single line and the correct result shall be written down, it shall never be erased; it shall also be dated and signed by the person making the correction.

10.2. Procedures for retaining information

The forms, databases and reports generated will be retained (printed and on optical or magnetic media) at the CIGB Clinical Research Direction (Documentation and GCP Group) for at least 15 years. The primary documentation generated in the study, held by the responsible investigator at each clinical site, should be retained for the same period of time.

The final report of the study will be made analyzing each of the results obtained in the statistical processing. This information is of a "restricted" nature, therefore only the researchers participating in the study will have access to it, and they are responsible for its care and conservation.

10.3. Data management and storage

For the purposes of this study, a data entry system will be generated in OpenClinica, which is a free software platform for protocol configuration and design of case report files, allowing the capture, storage and electronic

management of data. OpenClinica is developed from the most prestigious standards to achieve high levels of interoperability with other services and platforms. Its modular architecture, transparency and collaborative development model offer great flexibility while allowing high-performance solutions and scalability.

Primary data entry will be performed remotely from the clinical site. The operators of the databases will be trained by the person responsible for handling the study data by the CIGB. They will be able to access these databases at the CIGB (with a user name and password) through the national health system network. For the debugging of errors, the data not in agreement with the data recorded in the original models will be corroborated so as not to cause confusion. A record of this activity shall be kept so that it can be traced in the event of inspections and/or national and foreign audits.

XI. STATISTICS

11.1. Number of subjects planned

The sample size is determined by the total number of cases needed to demonstrate the vaccine efficacy (VE) to prevent COVID-19. Assuming proportional risks over time with 1:1 randomization of vaccine and placebo, **a total of 151 cases of COVID-19 will provide 90% test potency and detect a 60% reduction in the risk rate (60% VE)**, rejecting the null hypothesis *H0 = VE ≤ 30*, with two interim analyses reaching 35% and 70% of the total number of cases to be observed using a unilateral O'Brien-Fleming boundary for efficacy and a log-rank statistical test with a unilateral false positive error range of 0.025. The total number of cases refers to the PP set (per protocol) accumulating at the latest 14 days after the third dose. There are two planned intermediate analyses in this study, which will be executed when approximately 35% and 70% of the total number of cases to be observed are reached. 4.8×10^4 participants shall be randomized considering the following conditions:

- The desired VE is 60% (with the lower limit of 95% confidence interval to reject the null hypothesis H0 = VE ≤ 30%).
- An incidence rate of 0.286% in 2 months for the placebo arm.
- A dropout rate of participants of 2%.
- Two interim analyses reaching 35% and 70% of the total number of cases using a unilateral O'Brien-Fleming border for efficacy monitoring.
- 14 days of uniform recruitment.
- "Per Protocol" exclusion of 15% of participants, and subjects are at risk of COVID-19 14 days after the third dose.

The following table summarizes the conditions and sample size required to demonstrate the VE against COVID-19 with a 90% potency.

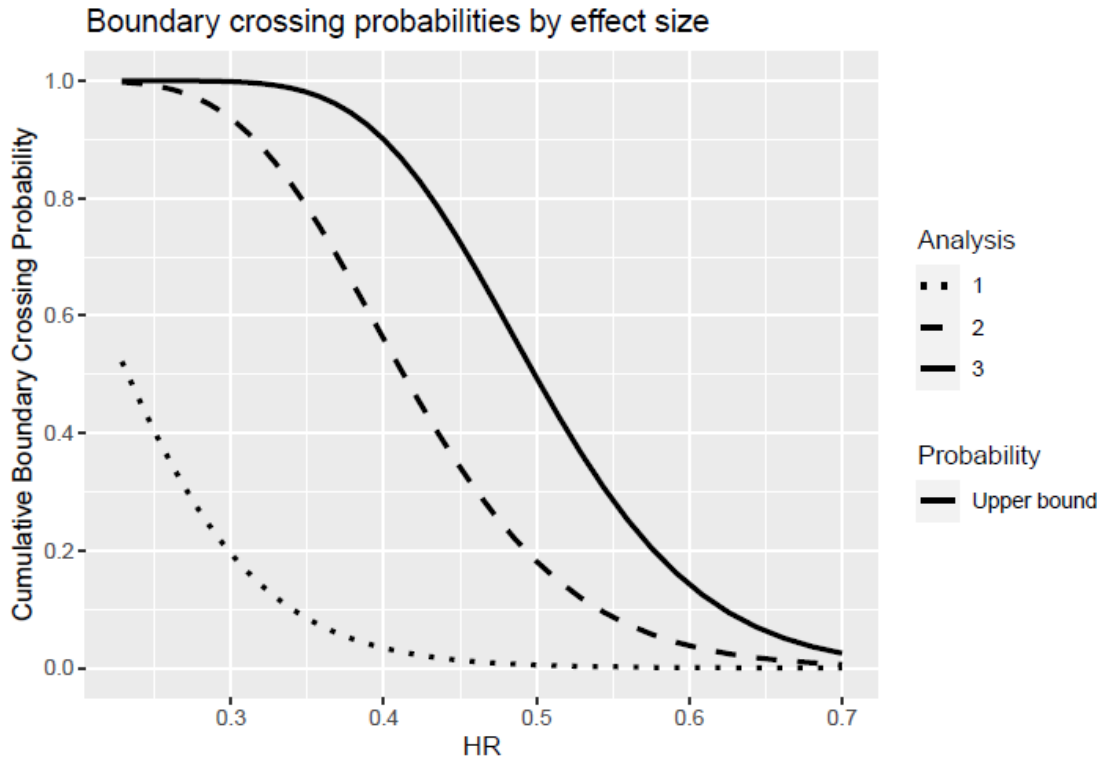
Desired VE	Lower limit	Randomization ratio	Events	Incidence rate in two months		Sample size
				Placebo	Vaccine	
60%	30%	1:1	151	0.286%	0.172%	48000

The sample size was calculated using the R gsDesign package.

Assuming an incidence rate of 0.286% in the placebo group, with 4.8×10^4 participants, it will take 1.47, 2.72 and 4 months from the application of the complete vaccination protocol to the first subject respectively to accumulate

35% (approximately 50), 70% (approximately 100) and 100% (approximately 150) of the number of events to be observed in the "per protocol" set.

La figura siguiente muestra la potencia de la prueba para rechazar la hipótesis nula con el valor de VE deseado ($VE = 60$ o $HR = 40$) en los dos análisis interinos y el análisis final asumiendo un total de 151 eventos.



11.2. Statistical analysis planned

11.2.1. Dataset analyzed

The following populations are differentiated:

***"Intention-to-treat"*:** all volunteers who have received at least one immunization. The individuals will be considered in the group where they were randomized.

"Modified Intention-To-Treat Set" (mITT): It is the subset analyzed by "intention-to-treat" that showed no immunological or virological evidence of previous COVID-19, at the time of inclusion in the study, before the first dose of the investigational product.

***"Per protocol"*:** defined as the included volunteers, who meet all selection criteria and mITT, who have received the full immunization schedule, in which the primary endpoint assessment is available and who have not undergone any major protocol deviations. The individuals will be considered in the group where they were randomized. All immunogenicity variables will be calculated in this population.

11.2.2. Exploratory analysis

With all the variables involved (primary, secondary and control), frequency distributions will be estimated in the case of qualitative ones and in the case of quantitative measurements of central tendency and dispersion (mean, median, standard deviation, interquartile range and minimum and maximum values).

The discontinuations will be analyzed through contingency tables with proportions and percentages; the Chi-square test or Fisher's exact test was used to assess independence from treatment and lists for causes will be made.

11.2.3. Confirmatory analyses

- **Efficacy:**

The efficacy analyses will be carried out with the *"mITT"* and *"per protocol"* sets and according to the group to which the participants were randomly assigned. The primary analysis will be performed using the "per protocol" population. The table below provides a summary of the methods to be used in the primary and secondary efficacy analyses.

Endpoints	Statistical methods
Primary endpoint: Efficacy of Abdala vaccine (VE) to prevent COVID-19.	- Primary analysis: VE will be estimated with 1 - HR (Abdala vs placebo) using the Cox regression model based on the "per protocol" set with cases considered 14 days after the last dose. - Likewise, for the case of the mITT set. - The sensitivity analysis shall be used from the same form mentioned above for the "per protocol" set with cases considered right after the 3rd or 1st dose. - Analysis of subgroups. The primary efficacy analysis will be made in the age groups considered in the sample design. - Auxiliary analysis of estimated VE with 1 - ratio of incidence rates with 95% confidence interval. - Auxiliary analysis of the cumulative incidence of VE
Secondary endpoints:	Method of analysis similar that of the primary endpoint. For each the following will be done:

Prevention of mild forms Prevention of moderate/serious forms	- Estimation of Cox's proportional risk regression model with data from the "per protocol" set, with cases starting 14 days from the 3rd dose of investigational product. - Analysis for mITT set data. - Sensitivity analysis with cases taken right after randomization, after the first dose, 14 days after the first dose and immediately after the last dose. - Efficacy of the vaccine and 95% confidence interval based on the incidence of cases will be estimated with 1 - ratio of incidence rates, using all cases.

The concept of vaccine efficacy is defined as the percentage reduction in risk (hazard) of the primary endpoint (vaccine vs placebo). The VE was estimated using one minus the (*hazard ratio*) HR.

❖ **Safety:**

All safety analyses will be carried out with the Safety Package. All safety analyses will be presented by treatment group, unless otherwise specified. Safety and reactogenicity will be evaluated through a clinical review of the relevant parameters (See paragraph IX of adverse events).

The number and percent of participants with adverse events will be reported during the 14-day follow-up period after each dose. A 95% confidence interval will be calculated using the Clopper-Pearson method for the percent of participants with adverse events for each treatment group.

The number and percentage of all remaining variables related to adverse events will be reported and tabulated. Descriptive statistics will be calculated for the remaining safety parameters.

The following table shows a summary of the planned safety analyses.

Variable	Number and percent of participants	95% Confidence interval
Type of adverse event	X	X
Expected adverse event	X	
Unexpected adverse event	X	
Unexpected adverse event with medical intervention	X	
Unexpected adverse event related to treatment	X	
Expected adverse event related to treatment	X	
Discontinuation due to adverse event	X	
Grade 3 or higher adverse event	X	
Grade 3 or higher adverse event related to treatment	X	

❖ **Immunogenicity:**

The immunogenicity set shall be made up of all participants in which the zero-time sample is available, prior to the first dose, where total antibodies to SARS-CoV 2 have been determined and immunogenicity results are available after the immunization schedule has been completed (per protocol population). If the number of individuals meeting this condition is very high, a subgroup will be taken to form the immunogenicity set, taking into account feasibility and analytical capacity.

Secondary immunogenicity variables will be analyzed using the Immunogenicity Subgroup, according to the vaccination group and baseline serological SARS-CoV-2 status, unless otherwise specified.

The complete set of immunogenicity analyses will be described, including the variant of sampling individuals in the immunogenicity set to characterize immunogenicity of the vaccine by evaluating immunological correlates of risk and protection.

Data from the quantitative immunogenicity tests will be summarized for each treatment group using positive response rates and geometric means with 95% confidence intervals, for each time an evaluation will be performed. The data from the qualitative tests (producing a positive or negative result) shall be summarised by tabulating the positive response frequency for each test per group at each time where an evaluation is carried out. Immunogenicity analyses will focus on two key moments and the change in marker response between them: Day 1 before the first dose of the investigational product and Day 56 (28 days after the third dose).

For each treatment, levels of antibody titers with their corresponding 95% confidence interval will be reported at each time. Descriptive statistics will also be reported including median, minimum and maximum.

For each treatment, antibody MGT will be reported with a corresponding 95% confidence interval at each time. Descriptive statistics will also be reported including median, minimum and maximum.

Multilateral 95% confidence intervals will be calculated using the Clopper-Pearson method at each time after the first dose of the number and percentage of participants with an increase ≥ 2, ≥ 3, and ≥ 4 of SARS-CoV-2-specific antibody titers and participants with seroconversion due to the vaccination compared to the titers before the first dose.

Seroconversion due to participant-level vaccination is defined as the change from a state below LOD or LLOQ to a state equal to or greater than LOD or LLOQ, or at least an increase ≥ 4 in terms of neutralizing antibodies or antibodies with specific vaccine antigens in participants with pre-existing antibodies.

A covariance analysis (ANCOVA) model at each time point of the study will be used to estimate the MGT of specific antibodies and vaccine versus placebo with the corresponding multilateral 95% confidence interval, with treatment group and stratification variable as independent variables and titers before the first dose as a concomitant variable.

Endpoint	Methods for statistical analysis
Specific antibody titers-values	• MGT for each group (vaccine vs. placebo) • MGT estimated by ANCOVA model
Seroconversion	• Descriptive statistics • Binomial variables increment ≥ 2, ≥ 3, ≥ 4 and seroconversion due to vaccine - Clopper-Pearson method.

11.2.4 Interim analyses

Before the final analysis, two interim analyses are planned with the main objective of detecting early evidence in favor of VE being greater than 30%. Interim analyses (IAs) will be performed when approximately 35% and 70% of the total expected events to be observed across both treatment groups have accumulated for the main analysis. A standard group sequential design will be followed using the Lan-DeMets expense function for the O'Brien-Fleming limits to preserve the type I error ($\alpha = 0.025$) concerning the rejection of the null hypothesis ($H_0: HR \geq 0.7$ or its equivalent $VE \leq 0.3$) throughout the interim and final analyses.

There are no intentions to stop the study if early efficacy is demonstrated during interim analyses. If efficacy is demonstrated during an IA, the following IA and the main analysis will be considered valid and supportive of the robustness of the study. The statistician or group of statisticians in charge of performing the IAs will not be blind, will be independent and will be isolated from the personnel involved in the study. Throughout the trial the results obtained during the IAs will be reported in terms of compliance or non-compliance with the predefined criteria.

Table 1: Lan-DeMets O'Brien Fleming group sequential limits for interim and final VE analyses.

	First Interim Analysis	Second Interim Analysis	Final Analysis
Month*	2	3	4
Number of events (% of total events)	53 (35%)	106 (75%)	151 (100%)
Efficacy limits for Rejecting $H_0: VE \leq 30\%$	$HR \leq 0.2586$ $VE \geq 0.7414$	$HR \leq 0.4351$ $VE \geq 0.5649$	$HR \leq 0.5052$ $VE \geq 0.4948$

p-value	0.0002	0.0073	0.0227
Probability of Crossing the Limit If the True $EV = 30\%$	0.0002	0.0074	0.0250
Probability of Crossing the Limit If the True $VE = 60\%$	0.0463	0.6146	0.9000

*Months begin to count 14 days after the first of the last doses of the vaccine candidate or placebo.

The first IA will occur when 53 events have been observed, which corresponds to approximately 35% of the total expected events. Based on the Lan-DeMets expenditure function for the O'Brien-Fleming group sequential limits, the first IA is considered to have demonstrated EV if the p-value corresponding to rejecting the null hypothesis $HR \geq 0.7$ is less than 0.0002. This corresponds to an observed HR of 0.2586, which is equivalent to an VE of 0.7414.

The second IA will occur when 106 events have accumulated, which corresponds to 70% of the total expected cases to be observed. The trial shall be considered positive during the second IA if the p-value of rejecting the null hypothesis $HR \geq 0.7$ is less than 0.0073 according to Lan-DeMets O'Brien-Fleming group sequential limits. This corresponds to an observed HR of 0.4351 which is equivalent to an observed VE of 0.5649.

The main analysis will be performed when approximately 151 events are observed. The study will be considered to demonstrate VE in the final analysis if the p-value associated with the rejection of the null hypothesis HR ≥ 0.7 is $\boldsymbol{HR \geq 0.7}$ less than 0.0227. This corresponds to an observed HR of 0.5052, which is equivalent to an observed VE of 0.4948.

XII. SUPPLIES

Resources	Responsible	Quantity
CIGB-66 / Placebo	CIGB	144000
Samples for immunological determinations	CIGB	96000
Forms (various) for primary data collection	CIGB	50 000
Research protocol / Annexes (*Reproduction*)	CIGB	150
Laboratory material (reagents, various supplies)	CIGB / MINSAP	-
Other medical supplies	MINSAP	-
Logistics (*workshops / meetings; quality monitoring visits; inspections; transportation*).	CIGB	-

XIII. GENERAL SCHEDULE.

	Start	Completion
Coordination and preparation of the protocol	February 2021	March 2021
Approval of Research Ethics Committees	March 2021	
Approval by CECMED	March 2021	
Workshop and protocol start visit	March 2021	
Execution of the study	March 2021	July - August 2021
Processing and analysis of final results	August 2021	
Drafting of final report	August 31, 2021	

XIV. PRACTICAL CONSIDERATIONS

14.1. Assignment of the duties and responsibilities in the protocol

14.1.1. Sponsor's Duties (CIGB)

1. Supply the product under study with the quality certificate and guarantee the rest of the resources.
2. Promote the clinical trial among qualified researchers and in a hospital that has the necessary conditions for its execution.
3. Designate the Monitor of the clinical trial, who will be responsible for the study as representative of the Sponsor's interests.
4. Conduct a workshop to unify criteria and initiate, where the protocol proposal is presented and discussed.
5. Inform investigators about the chemical-pharmaceutical, pharmacological, toxicological and clinical properties of the product under study. However, this obligation shall not imply that the Sponsor must provide information that is readily available and/or has been published and about which the Investigator could reasonably be expected to have knowledge in view of his or her professional background.
6. Provide, during the course of the clinical trial, any new information relevant to the conduct of the study that comes to their knowledge.
7. Retain all primary information and all research data for 15 years.

14.1.2. Duties of the monitor (CIGB, Clinical Research Direction)

1. Participate in the design and preparation of the Clinical Trial Protocol.
2. Submit the protocol to the centralized REC / SREC that is created for the purpose of the study.
3. Prepare technical file and feasibility report of the clinical trial and send to MINSAP to obtain approval of the study in the National Health System.
4. To register the study in the Cuban Clinical Registry of Clinical Trials, primary registry of the WHO.
5. To request the CIGB's Regulatory Department to process before the CECMED the authorization to start the clinical trial in Cuba.
6. Conduct quality monitoring visits during the execution of the study.
7. Immediately report to the Sponsor and CECMED the occurrence of any serious adverse event occurring in subjects participating in the trial.
8. Verify investigators' adherence to the approved protocol.
9. Inform the clinical site (and REC / SREC) about modifications to the protocol.
10. Participate in the preparation of the Final Report and publication together with the clinical investigators, product leaders and study advisors.

14.1.3. Duties of the Principal Clinical Investigator

1. Draw up the research protocol together with the study monitor.
2. Submit a copy of his *Curriculum Vitae* to the sponsoring center.
3. Participate in quality monitoring visits conducted by the monitor; ensure all documentation.
4. Submit the protocol to the REC / SREC for approval. Inform the latter if important situations occur during the course of the trial, such as the occurrence of serious adverse events.
5. Participate in the preparation of the Final Report and articles for publication.
6. Maintain the confidentiality of the information generated during the execution of the trial. In the event of any intention to disclose the results, whether preliminary, partial or total, the authorization of the sponsor must be obtained and all the considerations detailed in the section “Confidentiality considerations” must be complied with.
7. Verify compliance with the responsibilities of the co-investigators.
8. Retain documentation generated during the trial for 15 years.

14.1.4. Duties of responsible investigators/co-investigators

1. Know all the necessary information about the product under study, as well as its possible adverse events and be prepared to treat them in case they occur.
2. Participate in the criteria unification activities to be carried out.
3. Submit a copy of his *Curriculum Vitae* to the sponsoring center.
4. Maintain up-to-date clinical trial documentation.
5. Ensure compliance with the protocol, ethical principles and GCP.
6. Include all subjects who meet the selection criteria.
7. Obtain written informed consent from subjects prior to the start of the study.
8. Have available all the information requested during on-site quality controls and check-up visits. Deliver completed CRFs to ensure the speed and quality of the study data entry process.
9. Report any serious adverse events to the Sponsor (Monitor) and REC / SREC (first 24 hours).
10. Maintain the confidentiality of the information generated during the execution of the trial. In the event that the results are to be disclosed with the prior agreement of the principal investigator, whether preliminary, partial or total, the authorization of the sponsor must be obtained.
11. Retain documentation generated during the trial for 15 years.

14.1.5. Dutie of the Clinical Site Direction

1. Ensure that the personnel who will participate in the investigation have the necessary time to perform the tasks of the investigation.
2. Be aware of the objectives of the test and the requirements necessary to ensure its quality.
3. Support researchers in all aspects of clinical data collection.
4. Ensure that no other research is being conducted in the same service that competes with the present one.
5. Support research, as well as the quality controls that are programmed.
6. Contribute to the communication between the researcher and the sponsor.

14.2 Behavior before protocol deviations, treatment discontinuations or withdrawals

Deviations from the aspects foreseen in the protocol will not be admitted if they have not been previously analyzed and authorized by the Ethics Committee of this protocol and the CECMED. In the event of any deviation (non-adherence or non-compliance with the protocol during the execution of the trial), this will be analyzed by the Steering and Organization Committee of the study, which will determine the action to be taken in order to prevent its recurrence. If a subject does not show up on the day of the 2nd or 3rd dose of the investigational product, an attempt will be made to locate the subject to find out the cause and ensure attendance at the clinical site and receive the corresponding dose, up to 48 hours after the scheduled date (after that time, the subject will not be able to continue in the study.).

14.3. Procedures for the flow of the documentation and drugs

The investigational product and the primary data collection forms (various) will be delivered / collected by the CIGB, directly through the study monitors. The monitors, in the quality check and monitoring visits, will collect the forms once the participation of the subjects in the trial has concluded. The Supply group of the CIGB Clinical Research Direction will be responsible for the adequate distribution of vaccines (CIGB-66 or placebo) and medical supplies, as well as for the collection of the investigational product (dispensed and non-dispensed).

The investigator shall have a report of the number of subjects included, the adverse events detected, the withdrawals from the study and the causes thereof, as well as any other relevant information during the course of the trial, for when requested during the quality monitoring visits. In **Annex 16** you will find the location of the personnel linked to the research to establish any type of communication in relation to the clinical study.

14.4. Legal considerations on confidentiality, disclosure of results and other legal aspects.

The ownership of the results and data obtained in the clinical trial will correspond exclusively to the Study Sponsor (CIGB), which reserves the right to use them for submission to the health authorities of any country. The responsible researcher at the clinical site is obliged to provide the Sponsor with the results of the tests and all the data obtained during the research. The responsible researcher also undertakes to respect the confidential nature of the results and data obtained during this clinical trial.

The publication and/or disclosure of any information generated in this study must be previously agreed with the Sponsor. In any case, the confidentiality of the personal data of the subjects involved will be guaranteed and the legitimate interest of the Sponsor will be protected, such as, for example, obtaining optimal patent protection, coordination in the submission of documents to the health authorities, protection of confidential data and information, etc. If the IGBC considers it necessary to postpone the publication or presentation proposed by the researcher, he must do it. If the CIGB believes the researcher favors an interpretation of the data that may damage the rights of the CIGB, the research shall, without compromising his scientific integrity, try to adapt his interpretation so as to meet the CIGB criteria. If the parties do not reach an agreement, the investigator shall include in such publication or presentation, the interpretation of the CIGB.

The sponsor will not enter into any outside agreements with the clinical investigators from which additional financial compensation or any other type of consideration is derived, except for the costs of meetings for the organization of the study, as well as those facilities that in the future the sponsor may have available for the disclosure of the results obtained from the study in scientific meetings and publications.

The corresponding Ethics Committee will be asked to approve the clinical trial protocol; its members will be listed there, as well as the date and signature of each one, as proof of their approval for the execution of the trial in the Health areas of their territories.

The official list of authorized signatures in the study, made up of the researchers who will participate in the clinical trial, as well as appointed and authorized support personnel (e.g., clinical research coordinators), will be collected. The signatories of this document will be the only ones authorized to manipulate and complete any primary document generated in the clinical trial. This list shall be accessible to Cuban and foreign auditors interested in the research.

Monitors designated by the sponsor may access clinical information and documentation on the individuals included in the study for the purpose of verifying the accuracy and reliability of the data provided by the clinical researchers,

but do not have to collect personal identifying data on the study subjects. The clinical site will also facilitate access to this data to inspectors of the competent health authorities.

14.5. Quality Assurance Plan

Monitoring will be carried out at regular intervals, at least every two weeks, depending on the progress of the research, prioritizing those sites that require more attention from the CIGB. The objective of the control visits is to verify adequate compliance with GCP/adherence to the protocol and thus verify the efficient execution of the research. These visits will also be used to discuss any aspect of the protocol suggested by the investigator. These visits will be conducted by the monitors of the sponsoring center.

Each visit will be announced in advance. The researcher and the Health unit to which he belongs will allow the CIGB monitors to inspect and review the trial documents, including the use of the resources provided. The principal clinical investigator must have all the study documentation prepared and available for review. The aspects to be monitored and controlled were: compliance with the provisions of the protocol, record of included and not included subjects, storage conditions and use of the product under study, destination of the resources supplied, accuracy of data collection and safety profile (adverse events).

Inspections by the national authority (CECMED) may be carried out at any time during the trial.

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