Phase 1	Placebo	RBD 25 µg	RBD 50 µg	p value*
Schedule 0-14-28 days				
Geometric mean titers of RBD-IgG antibodies (95% CI)				
Day 42	1·95 (1·95-1·95)	22·35 (13·07-38·21)	131·2 (70·65-243·61)	**<0·0001**
Day 56	1·97 (1·93-2·02)	22·23 (13·09-37·75)	155·18 (84·12-286·27)	**<0·0001**
Inhibition to RBD-ACE2 binding Mean ± SD (95% CI)				
Day 42	1·84 ± 3·95 (0·08-3·59)	36·48 ± 26·54 (24·4-48·55)	65·84 ± 30·21 (52·44-79·23)	**0·008**
Day 56	6·36 ± 8·62 (2·54-10·19)	36·6 ± 24·54 (25·43-47·77)	75·71 ± 24·89 (64·67-86·74)	**<0·0001**
Geometric mean of neutralizing antibody titers against live SARS-CoV-2 (95% CI)				
Day 42	-	18·20 (10·72-30·91)	22·8 (12·19-42·64)	0·86
Day 56	-	10·4 (6·83-15·84)	31·53 (19·66-50·59)	**0·0032**
Schedule 0-28-56 days				
Geometric mean titers of RBD-IgG antibodies (95% CI)				
Day 56	2·09 (1·84-2·38)	12·21 (5·99-24·9)	16·11 (7·30-35·56)	1·00
Day 70	2·07 (1·87-2·3)	72·99 (39·84-133·73)	221·43 (127·56-384·37)	**0·023**
Inhibition to RBD-ACE2 binding Mean ± SD (95% CI)				
Day 56	4·28 ± 5·29 (1·93-6·62)	34·28 ± 26·22 (22·65-45·90)	37·56 ± 32·51 (23·14-51·97)	1·00
Day 70	6·85 ± 5·49 (4·42, 9·29)	72·32 ± 28·15 (59·84-84·8)	82·66 ± 19·37 (74·07-91·25)	0·35
Geometric mean of neutralizing antibody titers against live SARS-CoV-2 (95% CI)				
Day 56	-	17·41 (2·69-112·5)	31·09 (8·89-108·7)	0·37
Day 70	-	34·63 (18·05-66·46)	71·31 (38·57-131·83)	0·097

RBD: receptor binding domain. ACE-2: angiotensin-converting enzyme. ±SD: plus minus standard deviation. Data are n/N (%; 95% CI). Days 42 and 56, refers to 14 and 28 days after the third dose of the 0-14-28 days vaccination schedule and days 56 and 70, refers to 28 days after second dose and 14 days after the third dose of the 0-28-56 days vaccination schedule, respectively. * p values are for comparisons between 25 µg and 50 µg groups.

Table 1. Phase I Quantitative variables of immunogenicity in the three study groups by schedule and days

Age-stratified results (groups 19 to 54 years and 55 to 80 years) are shown in Table 2 and Figure 1.
At day 42, in the age group of 19-54 years, the seroconversion percentages of anti RBD-IgG were higher, 87% for 25 µg group and 94·7% for 50 µg group (p=0·036) and were lower in the older participants, age 55 to 80 years, 67·7% and 81·3% for the 25 µg and 50 µg group, respectively (p=0·052). The results at day 56 were similar. In the case of placebo group a small number of participants (less than 5%) seroconverted. The difference with respect to the placebo arm of the proportion of participants with seroconversion of anti-RBD IgG antibodies at day 56 was higher than 50% for all treatments arms. In the 19-54 age stratum, the differences were 78·2% (95% CI 70·6-85·9) and 88% (81·9-94), for the 25 µg and 50 µg, respectively. For participants between 55 and 80 years of age, the differences were 65·1% (53·7-76·4) and 79% (69·2-88·8), for the 25 µg and 50 µg, respectively. By day 42 and 56, differences between 25 and 50 µg groups were also obtained when the results were analyzed stratified by age, with higher GMTs for the 50 µg group and significant differences with the 25 µg group for both age strata and evaluation days (Figure 1A, 1D).
The media of the percentage of inhibition of RBD-ACE-2 binding showed an increase at 42 and 56 days, with respect to baseline levels for both 25 µg and 50 µg groups. Significant differences between both groups were obtained when the results were analyzed stratified by age, for both age strata and evaluation days (Figure 1B, 1E).
The proportion of individuals with Nab titers against live-SARS-CoV-2 was measured only at day 56, with no statistical differences found between 25 and 50 µg groups by the age groups of 19-54 years and 55-80 years, (p=0·72 and p=1·00, respectively). GMT of neutralizing antibodies were very similar for 25 µg and 50 µg groups, in the age group of 19-55 years (20·27 and 31·75) and for the age group of 55 to 80 years (35·51 and 28·98) without significant differences between them (p=0.052 and p=0.54 respectively) (Figure 1C, 1F).
In both age groups high seroconversion rates of anti RBD-IgG were developed, mainly in the 50 µg group, 93 3% (95% CI 88·0-96·7) for individuals from 19-54 years and 82·4 (73·0-89·6) for the older group of 55-80 years (p=0.015). No differences were found regarding the proportion if individuals with neutralizing antibodies to live SARS-CoV-2 (p=0.86) nor in the GMT of Nab, 31·75 (24·52-41·12) for individual of 19-54 years and 28·98 (20·75-40·46) (p=0.67).

Group	Age 19-54 years				Age 55-80 years			
	Placebo	RBD 25 µg	RBD 50 µg	p-value	Placebo	RBD 25 µg	RBD 50 µg	p-value
Seroconversion of anti-RBD IgG % (95% CI)								
Day 42	6 / 151 4% (1·5-8·4)	127 / 146 87% (80·4-92)	142 / 150 94·7% (89·8-97·7)	**0·036**	2 / 88 2·3% (0·3-8·0)	63 / 93 67·7% (57·3-77·1)	74 / 91 81·3% (71·8-88·7)	0·052
Day 56	8 / 151 5·3% (2·3-10·2)	122 / 146 83·6% (76·5-89·2)	139 / 149 93·3% (88·0-96·7)	**0·015**	3 / 88 3·4% (0·7-9·6)	63 / 92 68·5% (58·0-77·8)	75 / 91 82·4% (73·0-89·6)	**0·044**
Geometric mean titers of RBD-IgG antibodies (95% CI)								
Day 42	2·23 (2·06-2·42)	47·84 (36·51-62·69)	109·86 (83·23-145·01)	**0·0001**	2·16 (1·96-2·39)	29·76 (19·45-45·52)	71·29 (46·92-108·33)	**0·014**
Day 56	2·14 (1·98-2·32)	40·58 (31·57-52·17)	98·32 (75·42-128·17)	**<0·0001**	2·10 (1·93-2·28)	27·22 (18·30-40·48)	57·45 (38·08-86·68)	**0·044**
Inhibition to RBD-ACE2 binding % (95% CI)								
Day 42	7 / 151 4·6% (1·9-9·3)	90 / 146 61·6% (53·2-69·6)	109 / 150 72·7% (64·8-79·6)	0·058	4 / 88 4·5% (1·3-11·2)	47 / 93 50·5% (40·0-61·1)	67 / 91 73·6% (63·3-82·3)	**0·0021**
Day 56	10 / 151 6·6% (3·2–11·8)	89 / 146 61·0% (52·5-68·9)	112 / 149 75·2% (67·4-81·9)	**0·013**	3 / 88 3·4% (0·7–9·6)	43 / 92 46·7% (36·3-57·4)	61 / 91 67·0% (56·4-76·5)	**0·0087**
Inhibition to RBD-ACE2 binding Mean ± SD (95% CI)								
Day 42	4·62 ± 9·42 (3·09-6·16)	43·23 ± 29· 60 (38·23-48·23)	55·31 ± 32·56 (49·87-60·75)	**0·002**	5·68 ± 11·92 (3·06-8·30)	37·98 ± 32·52 (31·13-44·83)	50·51 ± 30·87 (43·77-57·25)	**0·034**
Day 56	4·09 ± 9·81 (2·49-5·69)	40·07 ± 29· 58 (35·07-45·06)	57·03 ± 32· 83 (51·55-62·52)	**<0·0001**	3·33 ± 6· 29 (1·95-4·72)	31·79 ± 31· 25 (25·21-38·38)	47·35 ± 32·04 (40·35-54·35)	**0·003**
Neutralizing antibodies against live SARS-CoV-2 (95% CI)								
Day 56	-	40 / 42 95·2% (83·8-99·4)	98 / 100 98·0% (93·0-99·8)	0·72	-	18 / 19 94·7% (74·0-99·4)	48 / 50 96·0% (86·3-99·5)	1
Geometric mean titers of antibodies against live SARS-CoV-2 (95% CI)								
Day 56	-	20·27 (13·87-29·61)	31·75 (24·52-41·12)	0·052	-	35·51 (19·56-64·48)	28·98 (20·75-40·46)	0·54

RBD: receptor binding domain. ACE-2: angiotensin-converting enzyme. ±SD: plus minus standard deviation. Data is n/N (%; 95% CI) for seroconversion rates of anti-RBD IgG, proportion of individuals with inhibition to RBD-ACE2 binding and neutralizing antibodies to SARS-CoV-2. Geometric mean titers are shown with 95% CI. Inhibition to RBD-ACE-2 is shown in means ± SD and 95% CI. Days 42 and 56 refer to 14 and 28 days, respectively, after the third dose of the 0-14-28 days vaccination schedule. *p-values correspond to comparisons between 25 µg and 50 µg groups.

Table 2. Phase 2 immunological results by study groups, stratified by age (19-54 years and 55-80 years) at 42 and 56 days.

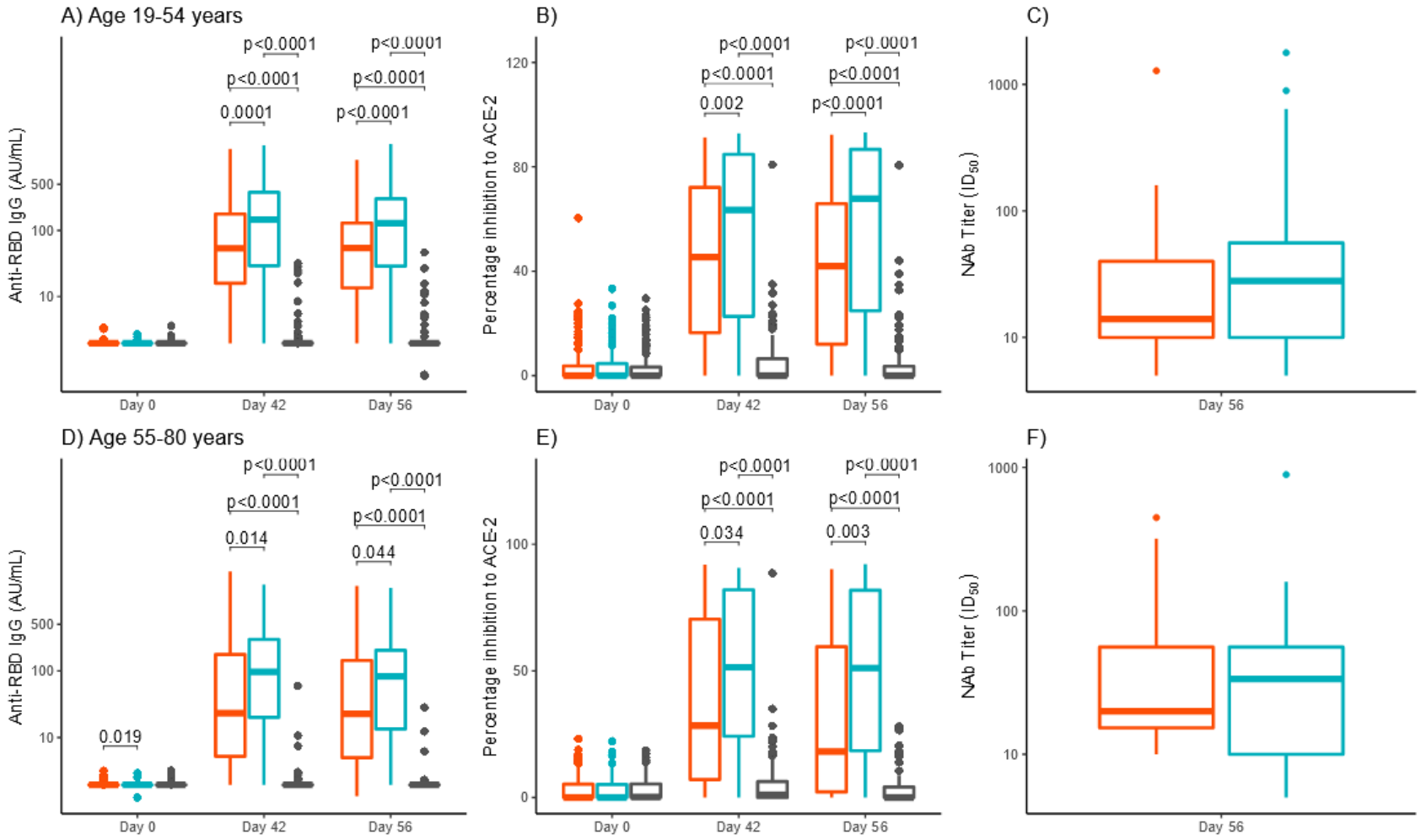

Figure 1. Phase 2 quantitative variables of immunogenicity by study groups and days.

Panels A and D: anti-RBD IgG antibody titers for age 19-54 years and 55-80 years, respectively. Panels B and E: Inhibition of RBD-ACE-2 binding for age 19-54 years and 55-80 years, respectively. Panels C and F: neutralizing antibody titers for age 19-54 years and 55-80 years, respectively. The study groups are represented by colors, red for RBD 25 µg, blue for RBD 50 µg and gray for placebo. The boxes and horizontal bars indicate interquartile range (IQR) and the median, respectively. The whisker's end points are the maximum and minimum values below or above the median ± 1·5 times the IQR. Points represent possible outliers. The braces contain the results of the Mann Whitney U multiple comparison tests with Bonferroni correction. For the viral neutralization variable, Student's t tests were used to compare geometric means. Only p values for significant differences are shown. RBD: receptor binding domain. ACE-2: angiotensin-converting enzyme. AU/mL: arbitrary units per mL. Nab: Neutralizing antibody titers.

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CIGB CENTRO DE INGENIERÍA GENÉTICA Y BIOTECNOLOGÍA

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BIOCUBAFARMA
GROUP FOR PHARMACEUTICAL AND BIOTECHNOLOGICAL INDUSTRIES

CLINICAL TRIAL PROTOCOL

CLINICAL STUDY "ABDALA"

VACCINE CANDIDATE CIGB-66 STUDY FOR ALL AIMING PREVENTION OF INFECTION OF SARS-COV-2

Evaluation of safety and immunogenicity of CIGB-66 vaccine candidate against SARS-CoV-2

Study Code: IG/CIGB-66I/CVD19/2002

Version 4.0

ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 presents an unprecedented challenge for Health systems around the world, Vaccines are required to face this health problem, hence clinical trials are currently being carry out with multiple vaccine candidates in order to obtain safe and effective preventive vaccines that manage to control this scourge, A phase I-II, monocentric, adaptive, randomized, parallel group, double-blind, placebo-controlled clinical trial will be carry out, with the primary objective of evaluating the safety and immunogenicity of the CIGB-66 vaccine candidate based on the recombinant RBD subunit. , administer intramuscularly (in two immunization schedules), in the prevention of SARS-CoV-2 infection, In phase I, 132 volunteers will participate and two strengths of the CIGB-66 vaccine candidate and two immunization schedules (0-14-28 and 0-28-56 days) will be evaluated, in a factorial design, which will end with an interim analysis for the selection of the two best experimental groups that fulfill the hypothesis of the study for this stage (safety and seroconversion of anti-RBD IgG antibodies to SARS-CoV-2), In phase II, 660 or 880 participants will participate (to be defined in the meantime) segregated into two age strata: 19-54 and 55-80 years, where the experimental groups selected in the first stage will be compared with a placebo, with a hypothesis of 50% superiority favorable to the CIGB-66 vaccine candidate compared to placebo in terms of seroconversion (main variable), Active surveillance of the biological safety profile will be carry out through the identification / characterization of adverse events (also main variable), As secondary variables during phase I, the following will be evaluated (on days 0, 42 and 56 for the short vaccination scheme; and 0, 56 and 70 for the long scheme): geometric mean of the specific IgG antibody titers anti-RBD and inhibition of the interaction of RBD with its receptor ACE2 by ELISA.

LIST OF ABBREVIATIONS USED AND DEFINITION OF TERMS

Abs: Antibodies

DNA: Deoxyribonucleic acid

Ag: Antigen

Intention-to-treat analysis: Strategy for analyzing data from a randomized, controlled trial, All participants are included in the branch to which they were assigned, whether or not they received or completed the intervention that was administer in that branch, This analysis avoids the bias caused by the loss of participants, which may alter the initial equivalence established by randomization and which may reflect a lack of adherence to the protocol, (Taken from the Cochrane Collaboration Glossary of Terms; version 4.2.5)

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

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

ECSR / ERC: Scientific Research Ethics Committee / Ethics and Review Committee

CIGB: Center for Genetic Engineering and Biotechnology

DCN: Data Collection Notebook.

COVID-19: Disease caused by SARS-CoV-2, from the English Coronavirus Disease 2019.

CTCAE: Common Terminology Criteria for Adverse Events

ELISA: Enzimed-linked immunosorbent assay

Adverse Event: It is any unfavorable medical incident that occurs in a person participating in a clinical trial before the administration of a pharmaceutical product, This incident is not necessarily causally related to the treatment, An adverse event can therefore be an unfavorable or unexpected sign (including an abnormal laboratory finding, for example), symptom, or illness temporarily

associated with the use of a medicinal product, (Taken from the Guidelines on Good Clinical Practices, CECMED, 2000)

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

ICH: International Conference on Harmonization

mg: milligram

MINSAP: Ministry of Public Health

RT-PCR: Real-time polymerase chain reaction.

RBD: Receptor-binding domain of the SARS-CoV-2 virus.

Adverse reaction: It refers to an adverse event that is considered causally related to the research product; includes overdose and drug interactions, Any unwanted harmful response produced by a pharmaceutical product at any dose should be considered an adverse drug reaction, A well-accepted definition of an adverse drug reaction is found in the Technical Report of the World Health Organization (Series No. 850, 1995) which states: "an adverse reaction is an unintended and harmful response to a pharmaceutical product which occurs at doses normally used in man for prophylaxis, diagnosis, therapy, or for the modification of a physiological function, In clinical trials, the damage caused by overdose, abuse or dependence, and interactions with other products, should be considered adverse reactions".

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

vs.: versus.

I. GENERAL INFORMATION

1.1. Title of the clinical trial: "Evaluation of Safety and Immunogenicity of CIGB-66 Vaccine Candidate against SARS-CoV-2". ABDALA Clinical Study

1.2. Code: IG/CIGB-66I/CVD19/2002

1.3. Sponsor:

- Center for Genetic Engineering and Biotechnology
  BioCubaFarma Biotechnology and Pharmaceutical Industries Group
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  ☎ (53-7)-2716022; Fax (53-7)-2716070 / 2736008; Web: http://www.cigb.edu.cu

1.4. Clinical site

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1.**5. Monitors - Direction of Clinical Investigations of the CIGB, Havana**

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MSc Yenima Martin Bauta: Graduate in Pharmaceutical Sciences; Master in Drug Research and Development; Associate Researcher; Assistant Professor of the University for Medical Sciences - Villa Clara.

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BSc Karen Urrutia Perez: Graduate in Pharmaceutical Sciences.

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1.7. Principal Clinical Investigator

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1.8. Participating researchers: See Annex 1

1.9. Responsible for the Conservation and supply of Medicines - CIGB, Havana

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Tech. Grettel Melo Suárez: Middle Technician in Computer Science; Innovative 1st Level Technician

Tech. Ketty Cruz Chirino: Middle Technician in Industrial Chemistry

1.10. Participants in immunological evaluations

From Direction of Biomedical Research - CIGB, Havana

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MSc. Gilda Lemos Pérez, Bachelor in Biochemistry, Master in Sciences in Current Trends of Biotechnology, Mention in New Products Research. Auxiliary Researcher

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Tech. Amalia Vázquez Arteaga Computer Technician.

BSc. Sheila Chávez Valdés, Bachelor Degree in Biochemistry.

Tech. Edelgis Coizeau Rodríguez, Chemical Analyst Technician.

BSc. Lismary Ávila Díaz, Bachelor Degree in Biochemistry.

Tech. Hany Lianet González Formental,Technician in Industrial Chemistry.

BSc. Ricardo Martínez Rosales Bachelor in Sciences in Microbiology.

Eng., Yahima Chacón Quintero, Chemical Engineer; Aspiring Researcher.

By the Civil Defense Laboratory (LISIDA), Havana

BSc.: María Teresa Pérez Guevara, Degree in Biochemistry. Associate Researcher

By the Direction of Research of the CIGB, Camagüey

DrSc Ana Cristina Campal Espinosa, Doctor in Veterinary Sciences; Auxiliary Researcher

MSc. Franklin Aguilar Fuentes: Master of Science; Assistant Researcher.

Tech. Lesvia Calzada Aguilera: Technical I level in Research, Innovation and Development.

1.11. Responsible for data management - Direction of Clinical Investigations of the CIGB, Havana

MSc. Patricia Lorenzo-Luaces Álvarez: Graduate in Mathematics; Master in Biostatistics; Added Researcher. Head of the Department of Statistics, Center for Molecular Immunology (CIM), Cuba.

Eng. Marel Alonso Valdés, Computer Science Engineer, Clinical Research Direction, CIGB.

1.12. Business Direction - CIGB, Havana

Dr.C. Miladys Limonta Fernández (Business Manager): Doctor in Technical Sciences; Chemical engineer; Master in Biotechnological Processes; 1st Level Technologist; Assistant Professor.

1.13. Logistic assurance - Direction of Clinical Investigations of the CIGB, Havana

BSc. Elizeth García Iglesias, Bachelor in Mathematics. Head of the Management and Control Group.

1.14. Ethics and Review Committee / Ethics Committee in Scientific Research

This clinical trial protocol will be submitted for the consideration and approval of the CER / ECSR of the Provincial Hospital. The approval opinion of this Committee will be annexed to the protocol that will be sent to the national drug regulatory agency (Center for State Control of Medicines, Medical Equipment and Devices - CECMED) for the authorization to start the study in the country.

II, INTRODUCTION

2.1. Fundamental information on the key problem and its context.

The global pandemic of the new coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in Wuhan, China, in December 2019, and has since spread throughout the world. the world [i,ii] As of November 21, 2020, 186 countries report the disease with 57,706,014 confirmed cases and 1,373,69675 deaths from COVID-19 (2.38% fatality), In the case of the Americas, the report to date is 24 694 469 confirmed cases and 699 296 deaths (2.83% fatality), while in Cuba the report is 7846 positive cases and 132 deaths (1.68% lethality[iii].).

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 likely originated from bat-derived coronaviruses with spread through an unknown mammalian host intermediate to humans[i], The SARS-CoV-2 viral genome is rapidly sequenced to allow diagnostic testing, epidemiological monitoring, and development of prevention and therapeutic strategies.

The clinical spectrum of a SARS-CoV-2 infection ranges from no 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, as well as fatigue and even diarrhea, In severe cases, the disease can progress rapidly, causing acute respiratory distress syndrome (ARDS), pneumonia, septic shock, irreversible metabolic acidosis, multi-organ failure, and bleeding disorders, among other complications, The prognosis varies from recovery in most cases to torpid evolution and death2,.

Proper management of COVID-19 requires a better understanding of the pathogenesis of the disease, Currently, there is no specific registered drug or vaccine for the SARS-CoV-2 coronavirus, and none have been fully evaluated for safety and efficacy.

Although the protection and social isolation measures that have been adopted by many countries have ensured that the majority of their citizens do not acquire SARS-CoV-2 infection, paradoxically they make these people more vulnerable when they face new waves of infection,, , Certain groups of

people are considered to be at high risk due to their higher morbidity and mortality, among them, the elderly and people with underlying diseases (high blood pressure, diabetes mellitus, heart disease, cancer, chronic obstructive pulmonary disease, among others) 6,.

There is consensus that, as long as there are no specific, safe and effective preventive vaccines for SARS-CoV-2, in sufficient quantities to implement global immunization programs, the world will not return to normal, 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 are currently being carry out in the world, with accelerated designs that overlap the traditional phases of clinical research, without breaching the Good Clinical Practice (GCP) standards, Obtaining safe and effective preventive vaccines, as well as their application with wide global coverage, would be the fastest and safest strategy to control this terrible pandemic10.

A fifth of the Cuban population is aged ≥60 years, according to the National Survey of Population Aging; of these, 58% suffer from high blood pressure, 16% suffer from diabetes mellitus, high blood pressure and thyroid diseases, and 19% have heart disease, A total of 86,000 people simultaneously suffer from three of these chronic diseases, increasing the chances of dying if they contract 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.

The Center for Genetic Engineering and Biotechnology (CIGB) in Havana, have been working on several vaccine candidates, using platforms already known by this institution and also considering the state of the art of research on 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 its active principle the recombinant protein RBD (Receptor-Binding Domain of the SARS-CoV-2 virus) obtain in the CIGB in *Pichia pastoris* and adjuvanted in alumina.

RBD component: Recombinant protein of the Receptor-Binding Domain of the SARS-CoV-2 virus, Rationale for the use of RBD as a vaccine antigen.

The choice of RBD as an active principle was based on the state of the art of newly acquired knowledge about the structure of the SARS-CoV-2 coronavirus and especially of protein S, or spike

protein, which is projected on the surface as a Common corona in this family of viruses and that is responsible for the name that identifies them, Protein S has a receptor-binding domain called RBD.

It is known that, in convalescents from COVID-19, most of the neutralizing antibody response against SARS-CoV-2, as well as a considerable portion of the cellular response against it, is directed against the spike protein (S) ,, being the perfect candidate for its inclusion in a vaccine preparation against said disease, supported not only by the specialized scientific literature on other coronaviruses such as SARS and MERS, but also by its inclusion as a vaccine antigen in the preparations that *Astra-Zeneca , Pfizer, Moderna* and other companies are currently evaluating,,.

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

Protein S is responsible for the interaction of SARS-CoV-2 with ACE2, its receptor in human cells25, This interaction occurs through a separate structural domain in this protein, known as RBD, which ranges from approximately cysteine 336 to cysteine 525, with a mass of approximately 25 kDa25, Although RBD shows some mobility with respect to the rest of the protein (it is found in two positions, the "*open*" and the "*closed*", which are important for interaction with the receptor), its structure is rigidly constrained by the presence of four disulfide bridges with a complex topology and the N-terminal end of the same is N-glycosylated in aspargins 331 and 343.

Many of the antibodies against RBD block its interaction with ACE2, thus neutralizing SARS-CoV-2; in fact, the epitopes recognized by a large number of the neutralizing antibodies directed against the spike protein are found precisely in the RBD. 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 to be satisfactorily evaluated in the clinic during the

development program of *BioNTech* / *Pfizer* vaccines, According to previous studies with SARS-CoV-1, the probability that immunization with RBD induces immunopathogenic phenomena is low17, and this molecule has been successfully expressed in a wide variety of systems ranging from mammalian cells (HEK293, CHO) to yeasts such as *Pichia pastoris*, Finally, the relatively rigid structure of RBD - stabilized by four disulfide bridges - confers a high thermotolerance to this antigen, which may give an advantage to vaccines based on this molecule in environments where it is reasonable to expect failures in the cold chain.

Although the RBD variant most studied in the literature is the one that goes from arginine 319 to phenylalanine 541, it leaves a strand at the N-terminal 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 promoting the formation of aggregates and intermolecular lattices. Extended variants such as 319-541 and related ones (318-510, 319-591) also have the disadvantage of being potentially less heat resistant and more sensitive to the action of proteases than shorter RBD variants (such as 331-528 and 331-532) due to the additional flexibility they bring to the ends of the molecule.

With the intention then 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 towards the N-terminal starting from the first cysteine of the domain to thus include the two N-glycosylation sites of the N-terminal end of the domain, which, according to previous data obtain for the RBD of SARS -CoV-1, are important for the expression of the domain in microbial hosts and four residues after the last cysteine extend through the C-terminal because there are several examples in the literature in which RBD is successfully expressed with this C-terminus. -terminal in microbial hosts 29,33.

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

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, More than 43 million

people infected and more than 1 million deaths from Covid-19 explain the growing negative potential of the Covid-19 pandemic for humanity, The resurgence of new COVID-19 cases in countries and territories where transmission control measures have been relaxed, suggests that only immunity, provide through vaccination programs, would control the spread of the virus or the fatal course of the disease in groups of risk, 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 financed by the governments of several countries have seen an unprecedented increase, This scenario allows many approaches to be tried and different platforms to be evaluated to develop specific immunity against SARS-CoV-2, and thereby obtain an effective vaccine candidate.

The development of neutralizing antibodies (AcN) 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 and Ebola, In the case of SARS-CoV-2, it is known that antibodies specific for protein S (on the surface) may have neutralizing activity, Protein S binds to the ACE2 molecule (human Angiotensin-Converting Enzyme 2) that mediates entry of the virus into target cells through its receptor-binding domain (RBD) located within the S1 subunit, For those antibodies that do not recognize RBD or recognize it with low affinity, a lower inhibitory potency of ACE2 binding and incomplete neutralization of virus entry are described, Thus, neutralizing antibodies that compete directly for binding to ACE2 are clearly the most effective, Consequently, the RBD was chosen as a target for the development of anti-Covid vaccines by many of the vaccine developers, In fact, it is known that the specific AcN response against RBD can generate a protective response in vivo in experimental animals challenged with the virus and prevent human cell lines from being infected in vitro, Furthermore, the use of recombinant vaccines based on RBD induces a high neutralizing activity in several species of experimental animals, including mice, rabbits and macaques, the latter having shown protection against viral challenge 43,45.

Within a vaccine development strategy, adjuvant selection is a key element in formulation development, Although many experimental adjuvants have been described, aluminum salts (Al^{3+}) are still the most widely used in parenteral vaccines, Depending on the nature of the antigen, this type of adjuvant can induce efficient humoral and cellular responses in the clearance of the virus and the inhibition of its entry into the host cell, as has already been demonstrated for strategies with a similar

design for the SARS-Cov virus., Aluminum salts are among the safest adjuvants, and it has been shown that exposure to aluminum in vaccinated children, according to the recommendations for vaccination programs, is low and is not easily absorbed by the body.

The projected strategy for CIGB-66 proposes adjuvant aluminum hydroxide of a fragment of the SARS-Cov-2 viral protein S (CRBDH6) produced recombinantly with a high degree of purity, Considering this, it was decided to carry out a series of research in experimental animals with the aim of developing an anti-Covid-19 vaccine candidate based on RBD that could be immunized by the parenteral route (i.e., intramuscular).

In the course of non-clinical experimentation for the vaccine candidate CIGB-66, the antigen CRBDH6 produced from the supernatant of HEK or CHO cells and subsequently from P pastoris was used as a base, For both variants of the protein, similar physical-chemical-biological characteristics were evidenced that support their use in an alternative way in the evaluation of immunization strategies in the development of a vaccine for SARSQov-2, In the studies, doses between 5 µg and 50 µg were used in BALB/c mice and in Non-human primates, which were administer in schedules of two immunizations with bi-weekly interval, including a third dose in some cases, In all the studies, ELISA-type assays were perform to study the induction of a specific IgG response for RBD using RBD-FcH (for mice) or RBD-FcM (for primates) in the coating, Likewise, the quality of this response was characterized by a competition test with the sera of RBD-FcH by ACE2-FcM (for mice) and of RBD-FcM by ACE2-FcH (for primates).

Evaluation of the adjuvant platform and immunization schemes for the generation of a specific immune response for the receptor binding site (RBD) of the S protein of SARS-CoV-2, in BALB/c mice, Use of RBD-FcM model antigen, (Assay 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 obtain 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 carry out by a parenteral route (ie, subcutaneous (SQ). For this, six BALB/c female mice of 6-8 weeks in each group (except group 10 which carry only three animals) by the SQ route in a 0.14 scheme, in a final volume of 100 µL, with the following groups: 1. RBD-FcM (20 µg) + HbsAg (5 µg); 2. RBD-FcM (5 µg) + HbsAg (5 µg); 3. RBD-FcM (5 µg) + HbsAg (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 for misfolded RBD protein and was obtain in E. coli.

The results obtain 13 days after the first dose showed 100% seroconversion with a geometric mean (GM) of the titers of 5,286 UStd/mL (standard units) in group 3 immunized with RBD-FcM (5 µg) + HbsAg (5 µg) in ALOOH which was significantly higher than group 2 inoculated with the same Ags without adjuvanting, which only had 50% seroconversion ($p < 0.0112$, unpaired t-test), Something similar was observe one week after the second inoculation (MG G3: 33524 USTD vs. MG G2: 80.25 USTD, $p = 0.0018$), This evidenced the importance of adjuvant in AlOOH by the parenteral route (**Figure 1A**).

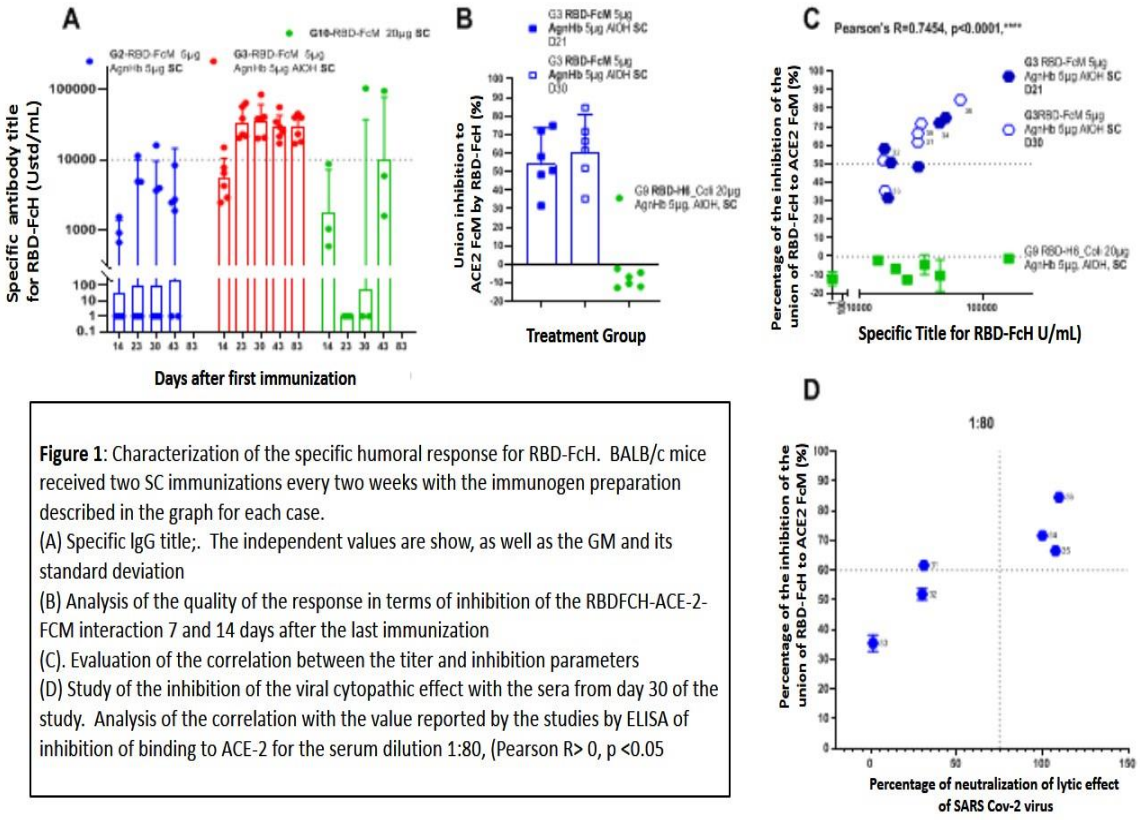


Figure 1: Characterization of the specific humoral response for RBD-FcH. BALB/c mice received two SC immunizations every two weeks with the immunogen preparation described in the graph for each case.
(A) Specific IgG title;. The independent values are show, as well as the GM and its standard deviation
(B) Analysis of the quality of the response in terms of inhibition of the RBDFCH-ACE-2-FCM interaction 7 and 14 days after the last immunization
(C). Evaluation of the correlation between the titer and inhibition parameters
(D) Study of the inhibition of the viral cytopathic effect with the sera from day 30 of the study. Analysis of the correlation with the value reported by the studies by ELISA of inhibition of binding to ACE-2 for the serum dilution 1:80, (Pearson R> 0, p <0.05

To verify the functionality of the RBD-specific antibodies, the sera were evaluated using an Inhibition Assay for the binding of RBD to its ACE2 receptor (SARS-CoV-2 virus receptor), Briefly, individual sera diluted 1:200 were incubated with one RBD-FcH molecule, Then, this mixture was added to a 96-well plate coated with the ACE2-FcM molecule, After washing, the bound RBD-FcH molecule was detect with a peroxidase-conjugated anti-human Fc, Finally, after extensive plate washes, the reaction was revealed by adding TMB substrate, The percent inhibition was estimated as the fraction between the OD in the wells incubated with the sera and the wells where the RBD-ACE2 interaction was maximum (without serum) expressed in percent.

As a negative control in this study, the serum of animals immunized with H6RBD expressed and purified from E. coli was used in order to corroborate the need for a correct folding of the antigen to obtain a quality response, Group 3 sera [RBD-FcM (5 µg) + HbsAg (5 µg), ALOOH] were evaluated in the RBD-ACE2 binding inhibition assay and compared with the response of group 9 sera. [H6RBD (20 µg)], As can be seen in **Figure 1A**, the animals that received treatment by the subcutaneous route (G3) showed the ability to inhibit RBD-ACE2 binding with respect to the group immunized with

the antigenic variant obtain in *E. Coli* (Group 9) that did not develop positive responses (**Figure 1B**), This result correlated with the specific antibody titer (R = 0.745, p <0.0001) (**Figure 1C**).

This result evidenced the need to produce vaccine antigenic variants for RBD in a system that replicates adequate folding to induce antibodies with possible neutralizing capacity, This inhibitory effect was also directly correlated with the inhibition of SAR*SQ*ov-2 viral infection in VERO-E6 cells (**Figure 1D**).

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

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

In order to explore the immunogenicity of an RBD expressed in the same cell line as used in previous experiments, but without the murine Fc region, an experiment was carry out in laboratory animals, In this study, the effect of increasing the dose on the generation of an anti-RBD humoral response in the serum of immunized animals was also proposed, For this, inoculations were carry out along the *SQ* route, with this antigen, Eight 9-10 week old female BALB/c mice were inoculated in each group by the *SQ* route, in a final volume of 100 µL or NAS, in a 25 µL volume, on a 0.14 day schedule, The groups inoculated by the *SQ*, according to the immunogen they were:

1) CRBDH6_HEK / AngHb (25 / 5µg) / ALOOH,

2) CRBDH6_HEK / AngHb (50/5 µg) / ALOOH,

5) CRBDH6_HEK (25 µg) / ALOOH,

6) CRBDH6_HEK (50 µg) / ALOOH,

7) HbsAg (2.5 µg) / ALOOH and AngHb (2.5 µg) (in) (negative control group).

The adjuvant with ALOOH was perform at 1.4 mg / mL. Inoculated by both routes in each dose.

The comparative analysis of the anti-RBD specific IgG titers in the serum of mice immunized by the *SQ*, between groups with equivalent amounts of RBD with HbsAg (G1: CRBDH6_HEK / AngHb (25/5 µg) / ALOOH and G2: CRBDH6_HEK / AngHb (50/5 µg) / ALOOH) and without HbsAg (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 immunostimulator HbsAg does not induce a differential response of IgG in the serum with respect to the groups without it, in the formulation, In order to better characterize this IgG response, the effect on the binding of RBD-FcH to ACE2-FcM in solution was evaluated (**Figure 2B**).

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

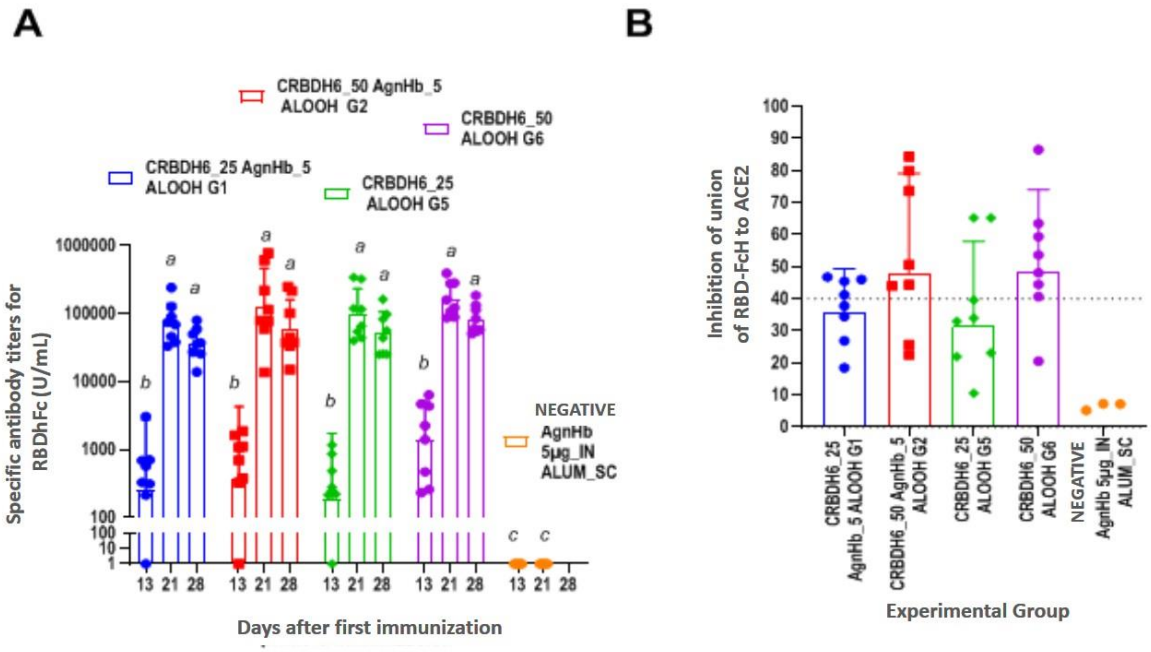


Figure 2. Humoral response of anti-RBD-FcH IgG, BALB / c mice were immunized twice by the *SQ* route, with the immunogen preparation described in the graph for each case, (A) Specific antibody titer studies, (B) Analysis of the effect of serum from immunized animals on the interaction of RBD-

FcH with the ACE2 FcM receptor, The independent data of each animal per group are shown in the form of points and the columns indicate the geometric mean.

The above results showed also in **Fig. 2**, indicated that the RBD protein produced in a cell line of human origin is highly immunogenic when inoculated parenteral using the aluminum hydroxide adjuvant. With only two doses, obtaining 100% seroconversion and the increase in antigenic concentration per dose led to a significant increase in the inhibition of ACE2 receptor binding.

Although the increase in the dose led to an increase in immunogenicity; the analysis of the data from **the CICUAL 20041 and CICUAL 20074** studies, as well as the recent literature regarding this type of subunit vaccine, indicated the existence of immunological reserves for the improvement of this response 45,. One strategy to achieve this effect is to increase the number of administrations that are carry out. In order to study the effect of an additional dose, the CICUAL 2006 study 2 was carry out.

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

To study the generation of anti-RBD humoral response in the serum of immunized animals, inoculations were carry out by a parenteral route (i.e., subcutaneous (*SQ*)) with the CRBDH6_HEK protein.

Therefore, to do that, 10 female BALB / c mice of 6- 8 weeks in each group by the subcutaneous route (*SQ*) in a three number administration scheme at 0, 14, 28, days in a final volume of 300 µL, with the following groups:

1. Placebo,
2. RBD (20µg)

The CRBDH6_HEK protein was adjuvanted in ALOOH at 1 mg / mL. The Placebo group was prepared the same as the rest of the immunogens, but without addition of the Ags. Ten days after the second and third doses, a positive response was observe in 100% of the animals. IgG titers in the serum of individual mice after the second dose showed a geometric mean GM=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 four times between doses (**Figure 3**).

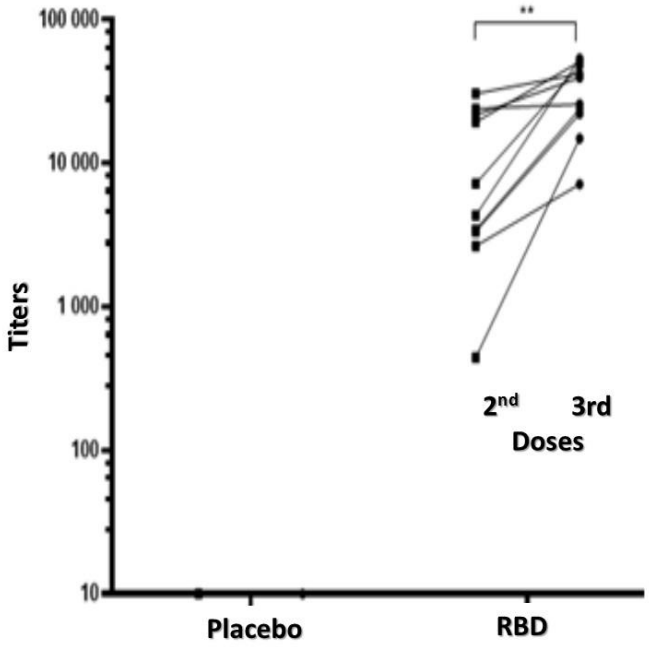


Figure 3. **Humoral response of anti-RBD IgG. BALB c mice were immunized three times by SQ way with**
1. **Placebo (AlOOH);**
2. **2. RBD (AlOOH).**

The sera were collected ten days after each dose and the titers were calculate by ELISA. The increase in titers between 3rd and 2nd dose was significant (p = 0.0019 paired t-test)

Regarding the quality of the response and in a similar way to what has already been describe it was observe that after two doses, half of the animals reached a percentage of inhibition of the binding of RBD to ACE2 greater 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 the values (i.e., Standard Deviation) was lower after the third dose (27.24 vs. 12.88) (**Figure 4**).

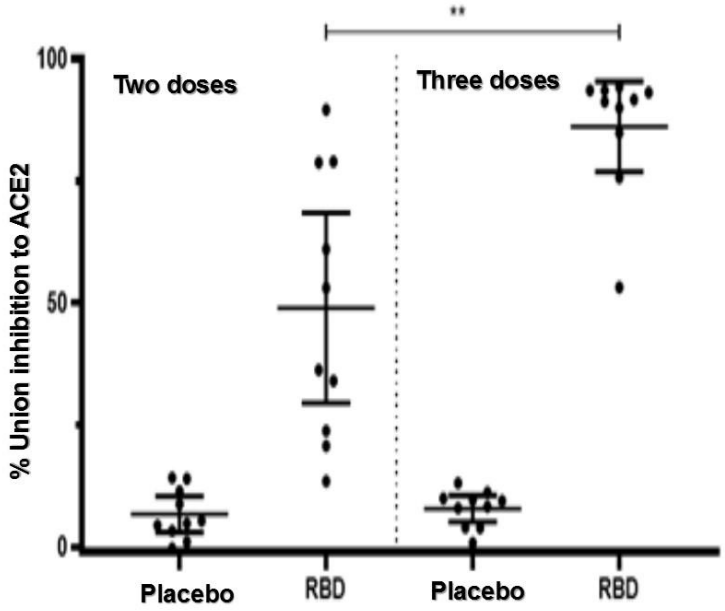


Figure 4. RBD-ACE-2 interaction inhibition assay
Balb / c mice were immunized two and three times via SQ with:

1. Placebo (AlOOH).

2. RBD (AOOH).

Sera were collect ten days after each dose and diluted 1: 200 for testing. The Wilcoxon test for paired samples showed a significant increase after the third dose vs. second dose (p = 0.0020).

These results confirmed those previously obtain after the second dose and indicated that the incorporation of a third dose to the scheme significantly increases the levels of potentially effective response already observed in the experimental animals.

Studies 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 observe in rodents, both in magnitude and in its kinetics 45,48. Therefore, it was consider appropriate to start two exploratory studies in Non-human primates (Green monkey, *Chlorocebus sabaeus*) with purified antigen from both types of host: *P. pastoris* (**CICUAL 20067**) and HEK/CHO (**CICUAL 20072**). These tests have as a fundamental objective the qualitative and quantitative analysis of the safety of the dose to be administer in humans, and of the kinetics of the immune response in a species that is closer to humans. The studies were adjust over time, in terms of dose frequency, to non-clinical observations in rodents.

In the **CICUAL 20072** study, the kinetics of the humoral immune response and its quality were analyze in groups of two animals with a mean age of 7 years.

Two experimental groups were evaluated that included

2.-. The intramuscular administration of 500 µL of 50 µg of CRBDH6_HEK adjuvanted in ALOOH (0.286 mg / mL) including 5 µg of HbsAg in the first two doses and in the re-activation dose;

3.- The administration of 50µg of total CRBDH6_HEK, divided between the two routes:

a) 100µl by intranasal route of 25µg of CRBDH6_HEK accompanied by 2.5µg of HbsAg in the first two doses and 40µg of HbsAg in reactivation on the day 35 of the study and

b) 500 µL intramuscularly of 25 µg of CRBDH6 adjuvanted in ALOOH (0.286 mg / mL), including 2.5 µg of HbsAg in the first two doses and in the re-activation.

IgG vs. RBD-FcM		Experimental Animal 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					

Inhibition RBD-ACE2 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 has been observe at the systemic level in the four animals that received intramuscular doses, but a delay in the appearance of the response is detect when compared with the studies of the parenteral route in rodents. The neutralizing response reaches 50% on day 28 of the study for only one animal (11413). This is consistent with studies of other vaccines in humans and with ongoing clinical studies for protein S or RBD subunit variants of SARSQOV-2, which indicate that optimal times between vaccinations are above 21 days and on average 28 days are being worked on 48,49.

In the **CICUAL 20067** study the kinetics of the humoral immune response and its quality were analyzed in groups of two animals with a mean age of 2 years. Two experimental groups that received three doses, were evaluated in scheme 0, 14, 28:

1. Intramuscular administration of 500 µL of 50 µg of CRBDH6_PP adjuvanted in ALOOH (0.286 mg / mL) including 5 µg of HbsAg; and
2. Administration of 50µg of total CRBDH6_PP divided between the two routes: a) 100µl by intranasal route of 25µg of CRBDH6_HEK accompanied by 2.5µg of HbsAg in the first dose and 40µg of HbsAg in the second and third doses and b) 500 µL intramuscularly of 25 µg of CRBDH6 adjuvanted in ALOOH (0.286 mg / mL) including 2.5 µg of HbsAg.

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				

Inhibition RBD-ACE-2 1:200		**Experimental Animal Group**			
Time	**lation**	**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**				

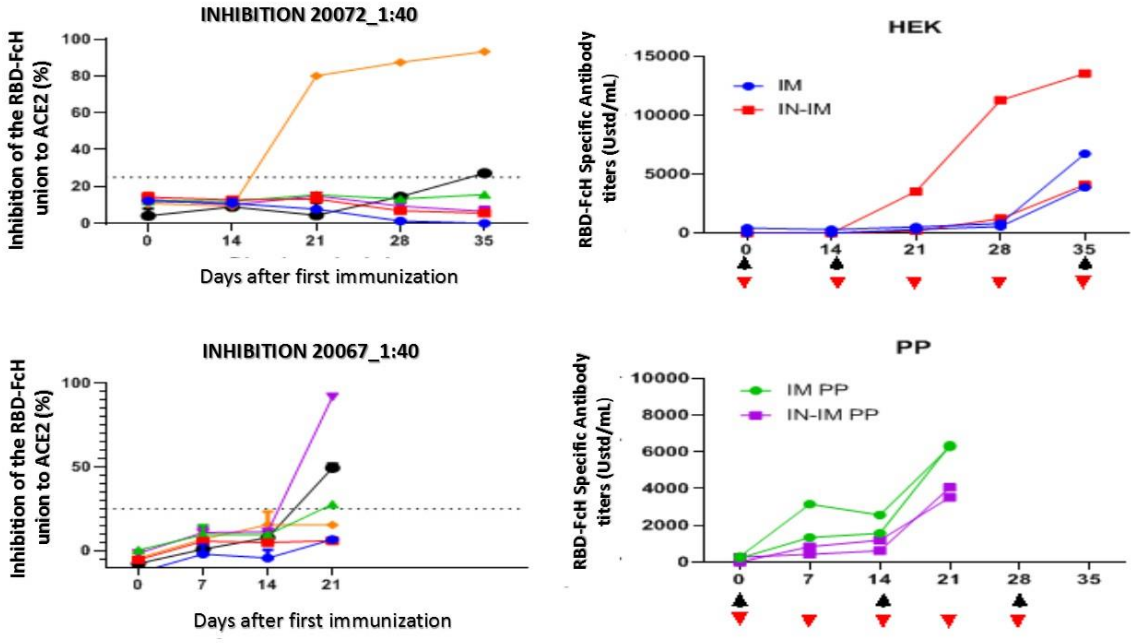
The experimental evidence presented showed that the CRBDH6 protein produced in human cells when adjuvanted in ALOOH, and administered by a parenteral route like subcutaneous (SQ.), promotes significant levels of serum IgG response. It showed in the test that this is functional response, and able to block *in vitro* the interaction with the SARS-CoV-2 virus receptor. It is known that the inhibition test predicts quite accurately the neutralizing capacity of sera from experimental animals44. It is important to note that, although parenteral immunizations with the recombinant RBD protein were only perform by the *SQ* route in mice, other groups have shown that the response levels for this route and the intramuscular route are equivalent in this species.

Furthermore, the results in Non-human primates indicate that by using the intramuscular route with the CRBDH6 obtains in *P pastoris*, a seroconversion of all the animals studied was achieve seven days after the second immunization.

In general, the results obtain with different RBD variants showed that there is a dose-response effect, in terms of the amounts of immunized RBD protein, which is reflected as a significant increase in anti-RBD IgG titers in serum and the ability to inhibit the RBD-ACE2 interaction. The same occurs with the increase in immunizations in terms of the quality of the humoral response in serum.

Finally, although HbsAg acted as a good immunopotentiator for the nasal route, when it was co-administer with CRBDH6. Whereas when administer by the parenteral route at 5 μg doses, did not contribute significantly to the increase in anti- RBD specific IgG titers, nor to their qualitative ability to inhibit the RBD-ACE2 interaction.

The studies carry out to date in Non-human primates show that, as has happened for other vaccine candidates against viral entities, the response in this species as well as in humans requires longer intervals in the immunization schedules, in order to establish an effective protective response.



So far, effective seroconversion has been observe at the systemic level of the four animals that received intramuscular doses from the seven days after the second immunization with kinetics similar to the Non-human primate 11413_3 of the **CICUAL 20072** study. However, in this experimental time only it was observe neutralization greater than 40% in an animal. Although earlier increases in seroconversion were detect for the antigens obtain from P pastoris, these results also point to the need for schedules with intervals greater than 14 days.

Clinical History

To date, around fifteen clinical trials of vaccine candidates based on protein subunits to prevent COVID-19 are publicly registered. At present, this is the most common platform for evaluation among vaccine candidates. Most of these vaccines contain the entire SARS-CoV-2 “S” protein, or portions of it like 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 effectiveness 6,,,.

One of these protein subunit candidates currently in Phase I / II clinical trials is Finlay-FR-1, sponsored by the Finlay Institute for Vaccines (IFV). This candidate is base on recombinant virus receptor binding domain (RBD) protein with adjuvant. The study is stratified into two age groups, a group of 19-59 years and another of 60-80 years and has the objective of 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 intramuscularly52.

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 the volunteers for a year and is expect to end in mid-2021.

This same company is sponsoring a phase II clinical trial in Changsha, China, which will evaluate 900 healthy volunteers between the ages of 18 and 59. , the conversion of neutralizing antibodies; and another phase I / II clinical trial in Hunan province, China to evaluate the candidate's tolerability and immunogenicity in 50 adults over 60 years of age53.

For its part, Novavax sponsors three clinical trials with its vaccine candidate NVX-CoV2373 of protein subunits, in this case the recombinant nanoparticle of Peak Protein of SARS-CoV-2. The first one is a multicenter phase I / II study, taking place in Australia and the United States to evaluate the safety and immunogenicity of this vaccine candidate with and without the MATRIX-M™ adjuvant in 1419 healthy volunteers between 18 and 84 years old. Another phase II study with the candidate NVX-CoV2373 adjuvanted with MATRIX-M™ evaluates the efficacy, immunogenicity, and safety of 2904 adults between 18 and 84 years with and without HIV infection. The third study is a phase III to evaluate the efficacy and safety of the candidate NVX-CoV2373 adjuvanted with MATRIX-M™ in 9000 SARS-CoV-2 serologically negative volunteers from the UK53.

Other pharmaceutical companies have formed strategic alliances in obtaining vaccines against SARS-CoV-2, such as the joint sponsorship of Clover Biopharmaceuticals Inc./GSK/Dynavax.

The phase I trial evaluates three dose levels (3, 9 or 30 µg), in schedules of 1 to 3 doses of the candidate of the trimeric Peak Protein subunit similar to the native protein, with and without adjuvant (AS03 or CpG 1018 more alumina) in healthy volunteers from Australia53,

The pharmaceutical company Sanofi Pasteur and GlaxoSmithKline also joined forces to develop a vaccine candidate based on protein subunits, in this case recombinant protein S with and without

adjuvant. This is a phase I / II clinical trial in 440 healthy adult volunteers from the United States, to be concluded by the end of 202153.

2.2. Purpose of the study

The COVID-19 pandemic caused by SARS-CoV-2 presents an unprecedented challenge for Health systems around the world. Vaccines are required to address this global health problem, which is why many clinical trials are currently being carry out 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 yeast *Pichia pastoris* as a model for the expression of recombinant proteins;
b) Having obtain in several expression systems, among them in CHO and yeast *Pichia pastoris*, an RBD (patent pending)

Once has been demonstrate the safety and immunogenicity of CIGB-66 in preclinical and toxicological tests, in addition to its physical-chemical characterization and a high-throughput production scaling that complies with GMP, the evaluation in humans of this vaccine candidate corresponds.

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

III. OBJECTIVES

3.1. General Objective

- ❖ Evaluate the safety and immunogenicity of the CIGB-66 vaccine candidate administer by the intramuscular route in the prevention of SARS-CoV-2 infection.

3.2. Specific objectives

- ✓ Identify and describe the adverse events that may occur after intramuscular administration of the vaccine candidate CIGB-66.
- ✓ Evaluate strengths and immunization schemes of the vaccine candidate CIGB-66, in terms of seroconversion of anti-RBD IgG antibodies to SARS-CoV-2 and inhibition response of the interaction of RBD with its ACE2 receptor.
- ✓ Evaluate the immune response to vaccination in terms of geometric mean of the titers of specific anti-RBD IgG antibodies and

3.3. Work Hypothesis

Phase I:

It is expect to identify the two best experimental groups (CIGB-66 dose level), regardless of the immunization schedules under study, which will continue to the second phase considering:

a) The percentage of seroconversion of anti-RBD IgG antibodies to SARS-CoV-2 (antibody titer ≥ 4 times the initial determination);
b) The occurrence to participants of Serious Adverse Events, in less than 5%, with a causal relationship attributable to the research product

Phase II:

It is expect, in at least one of the selected experimental groups:

a) in terms of number of participants a seroconversion of anti-RBD IgG antibodies to SARS-CoV-2, with 50% superiority compared to values of placebo group;
b) The occurrence to participants of Serious Adverse Events, in less than 5%, with a causal relationship attributable to the research product

IV. MEDICAL DEONTOLOGY

4.1. Ethics and Review Committee (ERC) / Ethics Committee in Scientific Research (ECSR)

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

- ✓ It conforms to the *Declaration of Helsinki* (Ethical principles for medical research on human beings, adopted by the World Medical Assembly, Fortaleza, Brazil, 2013). The study will be publish in the *Cuban Public Registry of Clinical Trials* (WHO primary registry) before the inclusion of the first individual.
- ✓ It complies with 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 well-being of the participants involved.
- ✓ It complies with the standards and minimum requirements established for obtaining consent.
- ✓ Describes the person selection criteria satisfactorily

4.2. Ethical aspects in conducting the trial

The clinical trial is duly endorse from the ethical point of view for the following reasons:

a. The clinical trial is approve by the CER / ECSR of the participating healthcare unit and the Cuban drug regulatory agency (CECMED). Likewise, as a contribution to the transparency of the investigation, the protocol will be visible in the Cuban Public Registry of Clinical Trials. Available at: http://registroclinico.sld.cu
b. Not occurrence in animals under toxicity studies, undesirable manifestations of the vaccine candidate CIGB-66 were find in the area of inoculation or at the systemic level.
c. There is evidence (in clinical trials) of the safety of the active ingredient and other components of the vaccine candidate. The investigational product is expect to have an adequate safety profile; however, in the event of the appearance of any adverse event, the

necessary procedures will be follow to counteract it, which may include the definitive interruption of the study.

d. The participants will be asked for their written consent, after knowing the possible inconveniences of the use of the product, the tests that will be carry out (of their importance and usefulness), as well as the necessary procedures for taking biological samples.
e. The study will be randomize, double-blind and contemplates the use of placebo, which is recommend in international guidelines for clinical trials of prophylactic vaccines. It is a clinical trial in exploratory stage, of a vaccine candidate for the prevention of a health problem (currently pandemic) that does not have any approved reference in the world (vaccine). Therefore, the use of placebo is justify and does not generate an ethical conflict. The volunteers to whom the placebo will be apply will have the possibility, if they accept it, to be vaccinated with the best strength and the scheme once sufficient evidence has been gathered, being a commitment of the promoter (CIGB).
f. After the trial, if the safety and immunogenicity of CIGB-66 is demonstrated in any of the immunization schedules that will be studied, immunization will be proposed to the participants of the ABDALA study who randomly received placebo or some CIGB-66 scheme / dose little successful.
g. All the work of care and evaluation of the participants included in the study will be carry out by trained medical and paramedical personnel.
h. The integrity of the participants will be respect, ensuring the confidentiality of their personal data.
i. An Independent Data Monitoring Committee (IDMC) will be create to review analysis and safety issues. Your suggestions will be take into account for decision-making.
j. All clinical investigators will be request to declare the absence of conflicts of interest.

4.3. Instructions for obtaining informed consent.

In order for an individual to be included in the trial, their informed, written, and signed consent must be obtain (**Annex 4**). During this process, all relevant information related to the study will be explain to the person so that he can freely decide whether he accepts to participate. Individuals will be informed of the right to participate or not and to withdraw their consent at any time, without exposing themselves to limitations on their medical care or other types of retaliation.

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

The researcher should obtain the person's written consent only after ensuring that he understood all the information provided. The procedure will be provide by means of a standard writing; in easily understandable language, (it should not be technical, but practical). Neither the investigator nor the trial staff can influence the person's decision to participate or continue in the trial.

The information to be provide to the individual must include at least the following:

- The study presupposes research.
- The objectives, immunizations, procedures to follow, 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.
- You will know if there is new information that may be relevant for the decision to continue participating in the study
- The expected duration of your participation in the trial, as well as the frequency and type of evaluations.
- Voluntary participation in the study and the possibility of rejecting or abandoning it without penalty or loss of the benefits to which they would have had access if they had not been included.

The person will write (in own handwriting) his name, the date and time of the granting of informed consent, likewise, he will write (in own handwriting) the name of the doctor who gave him the pertinent explanations; the latter will sign the document, A copy of the informed consent will be given to the individual (the original model will be kept at the clinical site, in the Investigator's Folder), If the participant cannot read or sign the document, an oral presentation will be made and the signature of his legal representative will be obtain, provide that he is witnessed by a witness not involved in the study and is mentioned in the same document.

No individual can be included in the study without having previously given their consent, The signature of the model by the person does not release the researchers, institutions or promoters from their obligations due to negligence.

4.4, Ethical responsibilities of all research participants.

Researcher: Adherence to the procedures established by the protocol and inform and request the consent of the participants.

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

Investigation team: Ensure compliance with assigned responsibilities.

Promoter: Guarantee the quality of the product under study, Monitor the execution of the study, verifying adherence to the protocol by the researchers and all the ethical aspects included in it.

Review and Ethics Committee: Review and approve the trial protocol ensuring the protection of the rights, safety and well-being of the participants involved in the study and provide a public guarantee of that protection, Verify the progress of the study and the adherence of the researchers to the protocol.

V. GENERAL CONCEPTION

5.1, Trial Design

A phase I-II, monocentric, adaptive, randomized, double-blind, placebo-controlled clinical trial will be carry out, with the primary objective of evaluating the safety and immunogenicity of the vaccine candidate CIGB-66 based on the recombinant RBD subunit, administer by intramuscular route (in two immunization schedules) aiming the prevention of SARS-CoV-2 infection.

Following an adaptive design, the study will combine exploratory phases I and II, with the possibility of selecting two experimental groups with the greatest possibility of success (which could correspond to different immunization schemes, or the same scheme), closing groups with high toxicity and even closure of the immunization scheme due to non-fulfillment of hypothesis in the 1st stage.

Phase I will be perform in individuals of 19-54 years of age and two strengths of the vaccine candidate CIGB-66 (RBD 25 and 50 µg) will be evaluated in two immunization schedules (0-14-28 and 0-28-56) in a factorial design, At the end of this phase, an interim analysis will be carry out to select the two best experimental groups immunized with the vaccine candidate CIGB-66 that meet the hypothesis for this stage (it may be one for each immunization scheme studied or even the two CIGB strengths- 66 of the same scheme).

For decision making, in addition, the responses of viral neutralization and inhibition of the interaction of RBD with its ACE2 receptor and anti-RBD MGTs will be considered, The state of the art will also be take into account in terms of possible findings in immune protection (benefit) or toxicity (risk) and finally practical factors such as the candidate that is acceptable in terms of safety and immune response, but that offers advantages. in its application or economic profitability.

In **Phase II**, the participants will be stratified into two age groups: 19-54 and 55-80 years, The experimental groups selected in the interim analysis will be compared with a placebo, Until this intermediate evaluation takes place, it will not be possible to know if one or both immunization schedules (short and / or long) will continue to be evaluated in the second stage.

The *Independent Data Monitoring Committee* will review and make periodic recommendations to the sponsor on safety, in the event of serious adverse events or other considerations associated with the

progress of the trial, It will also take into account the state of the art in terms of possible findings in immune protection (benefit) or toxicity (risk).

The clinical trial foresees the inclusion of:

- a **total of 1012 individuals** (**132 in phase I and 880 in phase II**), if in the interim analysis an experimental group is selected from each immunization scheme for the second stage.
- **a total of 792 individuals** (**132 in phase I and 660 in phase II**), if in the interim analysis the two experimental groups selected for phase II correspond to the same immunization scheme.

Individuals will be randomly assigned to one of the experimental groups (CIGB-66) or controls (placebo), in one of the planned immunization schedules (0-14-28 or 0-28-56), As it is a double-blind study, neither the individual, nor the physician, nor the analysts, nor the clinical trial monitors will know to which group they were assigned (the group will only be revealed at the end of the rigorous analyzes provide for in the protocol).

The study product (CIGB-66 or placebo) will be administer intramuscularly: 0.5 mL in the deltoid region.

PHASE I			PHASE II		
Study Groups	**N** (1:1:1)	**Scheme** (days)		**N** (1:1:1:1 o 1:1:1)	**Scheme** (days)
Group 1: RBD (**25 µg**) + Alum (0,30 mg) / 0,5 mL	22		The two best experimental groups vs. Placebo	220:220:220:220 o 220:220:220	**0-14-28 or 0-28-56, or one of these** (Provided they are for same scheme)
Group 2: RBD (**50 µg**) + Alum (0,30 mg) / 0,5 mL	22	0 – 14 – 28			
Group 3: **Placebo** + Alum (0,30 mg) / 0,5 mL	22				
Group 4: RBD (**25 µg**) + Alum (0,30 mg) / 0,5 mL	22				
Group 5: RBD (**50 µg**) + Alum (0,30 mg) / 0,5 mL	22	0 – 28 – 56			
Group 6: **Placebo** + Alum (0,30 mg) / 0,5 mL	22				

The vaccination process will be completed at the clinical site (by the nursing staff or researchers responsible for the activity), although the participants will be on an outpatient basis.

Time 0 will be considered the moment in which the person is included in the study (once the selection criteria have been confirmed) and receives the first dose of the product under study.

The medical team that executes the study will be responsible for all the medical care that the participants receive, from the moment they are received, for the duration of the clinical trial.

All measures will be take to ensure that the vaccination proceeds safely, For the safety assessment there will be an active surveillance of adverse events, For each dose there will be monitoring at the following times: **before** the administration of the product, **the first hour after** the inoculation of the product, **at 24 and 72 hours, and on the 7th day** (all personally at the clinical site during phase I, although during phase II a face-to-face consultation will not be necessary on the 7th day), but also at any other time when an adverse event occurs, volunteers will be instructed on the need to go to the doctor responsible for the study in the event of any medical eventuality. These observations include history, vital signs (temperature, blood pressure, respiratory and heart rates), inspection of the inoculation site for local signs or any other sign or symptoms and general physical examination.

Additionally, each individual will have an "*Adverse Events Card*" where they can record any sign or symptom that occurs outside the healthcare unit, which they will show to the clinical investigator for evaluation, This model will not constitute an obligation to fill out by the participants.

As part of the safety profile, all participants will undergo hematological determinations (hemoglobin, hematocrit, hemogram with differential, platelet count) and blood chemistry (glycemia, cholesterol, creatinine, uric acid, Glutamic Pyruvic Transaminase - SGPT, Transaminase Glutamic Oxaloacetic acid - SGOT) **at the beginning** (time 0, before the administration of the 1st dose of the research product) and 14 days after the last dose of the product (day 42 or 70, according to the short or long immunization schedule, respectively).

Immunological determinations (in serum) defined in the protocol will be carry out in the CIGB laboratories at the following times:

Groups with short scheme (0-14-28): **at the beginning, at 42 and 56 days**.

Groups with a long scheme (0-28-56): **at the beginning, at 56 and 70 days**.

5.2, Description of the Randomization Method

There will be a different random list in each phase of the ABDALA study, prepared by the *Supply Group of the CIGB Clinical Research Direction* with the support of 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 in two doses, or placebo) and immunization schedule (short or long).

- **FOR PHASE I:**

In phase I, only individuals aged between 19 and 54 years will be included and two immunization schedules will be studied (in a double-blind, placebo-controlled design):

a) short scheme (0-14-28) with two strengths of CIGB-66 (25 and 50 µg) vs., placebo.
b) long scheme (0-28-56) with two strengths of CIGB-66 (25 and 50 µg) vs., placebo.

The masking of the CIGB-66 (in its two strengths) and the placebo, will be guaranteed with the support of a random list in blocks of 12 individuals (**ratio 1: 1: 1: 1: 1: 1; 22 participants per group; 132 total participants**), This guarantees that, in a block of 12 individuals, two participants are included in each study group:

1) CIGB-66 25 µg;
2) CIGB-66 50 µg;
3) Placebo (the three in short immunization schedule: 0-14-28 days) and
4) CIGB-66 25 µg;
5) CIGB-66 50 µg;
6) Placebo (these last three in long scheme: 0-28-56 days), In turn, with this random distribution, in each block there will be six individuals in each immunization scheme (short and long).

The Supply Group will provide the monitors with the random list **without revealing to which study group (experimental or placebo) the volunteers have been assig**ned, They will only deliver a list indicating, for each inclusion number, the immunization schedule that each individual received (short or long); in this way, a homogeneous distribution of participants per scheme and per study group will be guaranteed (without losing the double-blind design), When the person is included in this 1st

phase, it will be registered in a consecutive order and in the row corresponding to the inclusion number, the immunization scheme (short or long) to which it has been assigned will appear.

FOR PHASE II:

In phase II, there is a need to stratify the participants into two age groups: 19-54 years and 55-80 years, seeking a balance between both strata. In addition, there is the peculiarity that, as a result of the interim analysis in response to the hypothesis proposed for phase I, the two best experimental groups (CIGB-66) will be selected, regardless of the immunization schemes under study, that could be selected for the second stage an experimental group from each evaluated scheme or the two experimental groups from the same immunization scheme (short or long). The randomization strategy for phase II will be depending on the decision making.

- **If an experimental group is selected in each immunization schedule:**

To achieve a balance between the two age groups for each vaccination scheme, the volunteers, as they are included in the study, will be wrote down in records segregated according to the age group to which they belong (19-54 or 55-80 years).

Each list will conclude upon completion of **396 participants in the 19-54 age group and 484 participants in the 55-80 age group**, so new volunteers corresponding to that particular age group will not be admitted, The difference between the totals of both age groups is due to the fact that, during phase I, 88 participants between 19-54 years of age were included in the groups that will continue to be active in the second stage, At the end of the clinical trial, there will be in both age groups the same number of participants assigned to the groups studied in the two phases (484 participants by age groups; 968 in total).

In the Registry of *Included* and *Not Included*, the participants recruited for the ABDALA study will be registered, in a consecutive order (from # 133, which is the one with which the 2nd stage begins) and in the row corresponding to the inclusion number it will appear the immunization scheme to which it has been randomly assigned (with a similar procedure explained for phase I: the Supply Group of the Direction of Clinical Research will indicate to the monitors, for each inclusion number, the vaccination scheme assigned to each person).

In this case, to guarantee the equiprobable assignment of the study groups, a random list will be drawn up in **blocks of 8 individuals** (ratio 1: 1: 1: 1)**: 880 participants in total (440 individuals in each vaccination scheme: 220 experimental and 220 controls**).

- **If the two experimental groups are selected in the same immunization schedule:**

To achieve a balance between the two age groups for each vaccination scheme, the volunteers, as they are included in the study, will be annotated in records segregated according to the age group to which they belong (19-54 or 55-80 years), Each list will conclude upon completion of **297 participants in the 19-54 age group and 363 participants in the 55-80 age group**, so no new volunteers corresponding to that particular age group will be admitted, The difference between the totals of both age groups is due to the fact that, during phase I, 66 participants between 19-54 years of age were included in the groups that will continue to be active in the second stage, At the end of the clinical trial, there will be in both age groups the same number of participants assigned to the groups studied in the two phases (363 participants by age groups; 726 in total).

In the Registry of *Included* and *Not Included*, the participants recruited for the ABDALA study will be registered, in a consecutive order (from # 133, which is the one with which the 2nd stage begins) and in the row corresponding to the inclusion number it will appear the vaccination scheme to which it has been randomly assigned (with a similar procedure explained for phase I: the *Supply Group of the Direction of Clinical Research* will indicate to the monitors, for each inclusion number, the vaccination scheme assigned to each person).

In this case, to guarantee the equiprobable assignment of the study groups, a random list will be drawn up in **blocks of 6 individuals** (ratio 1: 1: 1): **660 participants in total (220 individuals in each strength of the vaccine candidate CIGB-66 and 220 controls**).

In the Hospital Pharmacy of the Clinical site, there will be a “stock” with the masked research product (three doses per person with the medical supplies for its preparation / administration), in sufficient quantities to guarantee the advance of the protocol without interruptions for this concept, being the responsibility of the CIGB the continuous supply of vaccines candidates according to the inclusion rate.

At the time of inclusion (after verifying the selection criteria and obtaining the consent of the person), the clinical investigator (or failing that, the CIC) will contact the study monitor (representative of the promoting center - CIGB) who, after collection the general information of the person to be included will assign the inclusion number and the corresponding vaccination schedule (**Annex 7**). Given the characteristics of the study, the CIGB team of monitors will have an active presence at the clinical site that will carry out the clinical trial, in order to guarantee the necessary logistics, adherence to the protocol by clinical researchers and compliance with the GCP. In addition to facilitating the procedure for the inclusion of volunteers in the study.

Information on the identity of the study group to which each person was randomly assigned (CIGB-66 or placebo) will not be known to any of the investigators or the volunteers participating in the trial. The random lists will be kept safe under lock by the *Supply Group of the Direction of Clinical Investigations* of the CIGB.

5.3. Masking

Both the CIGB-66 vaccine candidate and the placebo have similar organoleptic characteristics, with no differences between them, which guarantees 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 researcher in the healthcare unit will contact the main monitor of the study who will be in charge of opening the code for that particular person, according to established procedures.

5.5, Identification of the participants

Each individual will be identified by a code that indicates the healthcare unit executing the study followed by the “inclusion number” (consecutive), This identification must appear in the documents corresponding to each person, The Provincial Hospital "Saturnino Lora" will be identified with the initials SL; therefore, the identification code of the 1st person will be: SL-001.

During the first phase, 132 participants (**SL-001 - SL-132**) will be included, In the second stage, the consecutive numbering from the previous stage (**from SL-133**) will be followed.

During phase I of this study there will be a PRE-inclusion (**Annex 12**), at which time informed consent will be obtain from the volunteers, as well as rigorous evaluations (clinical and laboratory) to verify the criteria of selection.

At this stage the participants will have a preliminary code (example: **PISL-001**), which will be consecutive in the same order in which they respond to the call for the clinical trial, This pre-inclusion number does not correspond to the official code that will be given to the person if he is finally included in the ABDALA study, In phase II no pre-inclusion check is foreseen.

5.6, Factors that can be introduced to reduce biases

The following actions, procedures or instructions will be established to eliminate or minimize errors and biases that prevent compliance with the GCP 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 medicines regulatory agency (CECMED) approves the trial, a start-up workshop will be held for the analysis, discussion and mastery of the protocol, in order to ensure adherence and compliance with GCP by all researchers.
- The study will be randomized in blocks to guarantee homogeneity in the number of participants included in each group and vaccination schedule, Likewise, the trial will be double-blind and controlled with placebo, methodological pillars in clinical research that provide rigor and scientific credibility.
- The main clinical investigator and the trial monitors will ensure that the administer research product is properly labeled and identified (**See section 7.5**) and corresponds to the one assigned in the random list, In addition, they will verify that the biological samples (serum) destined for immunological evaluations are properly labeled with a coding system (four-digit, random, which does not include the person's identification code or the sample extraction time) that guarantees the total blinding of the CIGB analysts.
- The product (CIGB-66 vials or placebo) + disposable syringe with needle for intramuscular administration will be provide by the *Supply Group of the CIGB Clinical Research Direction* in plastic envelopes individualized by each volunteer.

- Quality monitoring visits will be carry out at all stages of the trial, ensuring strict compliance with the provisions of the protocol.
- The study will have an *Independent Data Monitoring Committee*, which will review and make periodic recommendations to the promoter on safety, in the event of serious adverse events or other considerations associated with the progress of the trial.

VI, SELECTION OF PARTICIPANTS

6.1, Study universe

Constituted by those adult person, of any sex, permanent residents in the capital city of Santiago de Cuba province (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, Inclusion criteria

1) Individuals aged between 19 and 54 years (for phase I) and between 19 and 80 years (for phase II).
2) Physical examination without significant alterations like skin lesions that interfere with the safety assessment, clinically relevant alterations during the anamnesis or examination by devices, altered vital signs.
3) Hematological and blood chemistry determinations of values within or outside normal ranges, without clinical relevance (only for phase I).
4) Voluntariness of the person by signing the informed consent.

6.3. Exclusion Criteria

1) Virological diagnosis by RT-PCR, or other diagnostic procedure, of SARS-CoV-2 infection.
2) Contact or suspect of COVID-19 at the time of inclusion.
3) Individuals at high risk of exposure to SARS-CoV-2 infection [*contacts of confirmed cases, health workers in 1st line of medical care (Emergency, ICU, other risk areas*)].
4) Acute infection in the last 15 days or presence, at the time of inclusion in the study, of signs and symptoms such as: fever, cough, dyspnea or anosmia / ageusia.
5) Chronic, autoimmune or endocrine-metabolic diseases decompensated at the time of inclusion.
6) Body Mass Index ≤18 or ≥ 35; Kg / m2.
7) Individuals with tattoos in both deltoid regions.
8) Administration of any research product in the last three months.

9) Subject treated in the last three months or with any medical condition that requires an immunomodulator as interferon, transfer factor, biomodulin T, thymosin, others, and steroid (except topical or inhaled) or cytostatic during the study.
10) Have received blood, immunoglobulins or blood products in the three months prior to the start of the study.
11) Known hypersensitivity to thiomersal and to any of the components of the formulation under study.
12) History or suspicion of alcoholism or drug dependence.
13) Pregnancy or breastfeeding, woman of reproductive age not using contraceptives or planning pregnancy.
14) All women of childbearing age will be given (before inclusion in the study and each dose of the research product) a test for the early diagnosis of pregnancy in urine, using the HeberFast Line® MaterniTest kit (Heber Biotec, Havana , Cuba), If positive, the woman cannot participate in the study (or continue in it if it is detect after inclusion).
15) Obvious mental incapacity to give consent and act accordingly with the study.

6.4, Clinical Trial Stopping Criteria

- ✓ Clinical Trial stops due to Inadmissible serious toxicity greater than 5% with high probability (> 0.90) and proven causality (probable or definitive) attributable to innoculation with the vaccine candidate some experimental group.
- ✓ Clinical Trial will be stopped if there is no evidence of success in any experimental group during the planned interim analysis.

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 "Saturnino Lora" Hospital in Santiago de Cuba. They will be convened for the review of the intermediate analyzes and will accompany the evaluation of the primary information in order to make timely recommendations to the promoter.

The members of the Independent Data Monitoring Committee (IDMC) are:

- ✓ DrC. Hector Lazaro Lara Fernández *(Responsible of the Committee)*: Doctor of Medical Sciences; Doctor of Medicine; 1st grade specialist in Hygiene and Epidemiology*;* Master in Pharmacoepidemiology. National Coordinator Center for Clinical Trials (CENCEC), Havana
- ✓ DrC. Teresita de Jesus Montero González: Doctor of Medical Sciences; Doctor of Medicinnd; 2nd Grade Specialist in Anatomy Pathology; Master in Higher Educational Sciences. "Luis Díaz Soto", Central Military Hospital, Havana
- ✓ Dr. José de Jesus Rego Hernández: Doctor of Medicine; a de 2do Grade Specialist in Internal Medicine; Master in Pharmacoepidemiology. "Dr. Salvador Allende", Surgical-Clinical, Hospital, Havana
- ✓ Dr. Beatriz Amat Valdés: Doctor of Medicine, 1st grade Specialist in Immunology; "Luis Díaz Soto", Central Military Hospital, Havana
- ✓ Dr. Mayté Robaina García: Doctor of Medicine; 1st grade Specialist in Biostatistics; National Coordinator Center for Clinical Trials (CENCEC), Havana
- ✓ MSc. Dianne Yurien Griñan Semaná: Bachelor in Pharmaceutical Sciences; Master in Natural and Traditional Medicine, Associate Researcher; Instructor Professor. Provincial Coordinator of Clinical Trials in Santiago de Cuba province / CENCEC

6.5. Clinical Trial Exit Criteria

All participants, once they have been included in the study and regardless of later discontinuation, will be part of the statistical analyzes planned in the study, as appropriate. In the case of voluntary abandonment, an attempt will be made to obtain the cause of the abandonment and all the available information.

VII, TREATMENT

7.1, Products to be used

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

- **CIGB-66** (Composition per mL - NP 5317C).

Batches: RPQ120021 / 0 (25 µg) and RPQ020021 / 2 (50 µg), Both batches expire on 03/2021.

Composition	Components per mL	Quantity	Part No.
API	RBD	50 or 100 µg	4502
Aluminum hydroxide gel	(Al3 +)	0.60 mg	620
Disodium hydrogen phosphate	(Na2HPO4)	0.56 mg	008
Sodium dihydrogen phosphate dihydrate	(NaH2PO4 • 2H2O)	0.62 mg	119
Sodium chloride	(NaCl)	8.5 mg	023
Thiomersal	0.05 mg	292	
Water for injection	wpi	q.s.	185

Product Characterization and Control

The vaccine candidate against the SARS-CoV-2 virus by intramuscular route CIGB-66 is packaged in 2R vials for injections, DIN standard hydrolytic quality Type I, of clear crystalline color with a 13 mm gray chlorobutyl stopper and flip-cap seal.

Quality controls

- Organoleptic characteristics (Visual method): Slightly opaque white-grayish suspension that separates, after a sedimentation time, into two phases: one transparent liquid and the other in gel form, which when shaken is easily resuspended and is essentially free of particles foreign.

- Sterility (PPO(SOP) 4.09.274.941; PNO 01.5803; FE / USP): Passes the test.
- Pyrogens (PNO 07.0023; USP Dose in rabbit: 50 µg / Kg animal weight): Compliant.
- Immunogenicity (PPO(SOP) 4.09.642.201): ≥ 50% seroconversion
- Percent adsorption of RBD: Microcomassie method; PPO(SOP) 1.34.604.032): ≥ 50%
- Identity: (Western Blot; PPO(SOP) 4.09.470.151; PPO(SOP) 1.34.602.032): Identification of the majority band
- Aluminum Ion: (Complexometry; PPO(SOP) 4.09.064.921; PNO 01.3993): From 0.30 to 0.85 mg / mL
- Thimerosal: (Spectrophotometry; PNO 01.3993): 0.030 to 0.100 mg / mL.
- Volume: (PPO(SOP) 4.09.271.021; PNO 01.3993; USP): Not less than the volume declared on the label.
- pH: (PPO(SOP) 4.09.068.921; PNO 01.3993; USP): 6.00 to 7.00.

➢ **Placebo for intramuscular administration (**Composition per mL - NP 5319C) Batch: RPQ20011 / 0; expires: 03/2021.

Composition	Components	Quantity per mL	Part No.
Aluminum hydroxide gel	(Al3 +)	0.60 mg	620
Disodium hydrogen phosphate	(Na2HPO4)	0.56 mg	008
Sodium dihydrogen phosphate dihydrate	(NaH2PO4 • 2H2O)	0.62 mg	119
Sodium chloride	(NaCl)	8.5 mg	023
Thiomersal	0.05 mg	292	
Water for injection	wpi	q.s.	185

7.2. Route of administration, dose, frequency and duration of treatment

The research product (CIGB-66 or placebo) will be administer intramuscularly: 0.5 mL in the deltoid region.

PHASE I			PHASE II	
Study Groups	**N** (1:1:1)	**Scheme** (days)	**N** (1:1:1:1 o 1:1:1)	**Scheme** (days)
Group 1: RBD (**25 µg**) + Alum (0,30 mg) / 0,5 mL	22		The two best experimental groups vs. Placebo	0-14-28 or 0-28-56, or one of these (Provided they are for same scheme)
Group 2: RBD (**50 µg**) + Alum (0,30 mg) / 0,5 mL	22	0 – 14 – 28		
Group 3: **Placebo** + Alum (0,30 mg) / 0,5 mL	22			
Group 4: RBD (**25 µg**) + Alum (0,30 mg) / 0,5 mL	22			
Group 5: RBD (**50 µg**) + Alum (0,30 mg) / 0,5 mL	22	0 – 28 – 56		
Group 6: **Placebo** + Alum (0,30 mg) / 0,5 mL	22			

Innoculations of CIGB-66 or placebo will be completed at the clinical site Hospital SL (by the nursing staff responsible for the activity), although the participants will be on an outpatient basis.

For the administration of the test product follow the following instructions:

1. For the administration of the test product, each dose will require: a 2R vial (CIGB-66 or placebo, solution for intramuscular injection) + a 1 mL syringe with a 23G x 25 mm needle.
2. The administrations by intramuscular injection of CIGB-66 or placebo will be carry out in the external face of the upper part of the arm (preferably the non-dominant part of the person), in the deltoid region, according to the intramuscular injection procedure.
3. For each 0.5 mL 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 take to extreme during the intramuscular inoculation of the product (CIGB-66 or placebo) and for the elimination of the used material.

Important:

- Upon completion of intramuscular application, discard 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 promoting center and destruction according to the procedures established by the CIGB.
- Safety regulations, use of gloves, mask and eye protection must always be observe to avoid possible transmission of infections. Wash the hands following the biosafety stablished procedures.
- If there is a volume of the product remaining, it will be discarded, You must use all biosafety precautions to eliminate waste and to avoid possible contagion from the person applying the product and from other people.

7.3. Rationale of the dose used

The selection of the strengths and immunization schemes of CIGB-66 were based on the clinical results found in the literature with candidates also based on RBD protein subunits, Preclinical results with this protein were also considered.

There is a history of clinical trials for RBD conducted this year to face COVID-19 in which RBD concentrations range from 9 µg to 100 µg.

The pharmacological studies carry out, comparing strengths of 25 and 50 µg of RBD, show a better response when increasing the dose (report of pharmacological study CICUAL / CIGB / 20074).

The 248 vaccine candidates in development and the 49 already in clinical study include the RBD region. Of these candidates, 76 are subunit and those listed below, which include only RBD, are already in different phases of clinical studies.

- BioNtech Anhui Zhifei Longcom Biopharmaceutical (Subunit)
- West China Hospital, Sichuan University Phase I (Subunit)
- Anhui Zhifei Longcom Biopharmaceutical (Subunit)
- Clover Biopharmaceuticals (Subunidad)
- Kentucky Bioprocessing (Subunidad)
- Adimmune (Subunidad)

- Instituto Finlay de Vacunas (Subunidad)
- SpyBiotech (Subunidad)

Of these, the BNT 162b2 from BioNtech is an mRNA vaccine that encodes only the RBD region, began a phase I trial in the US with 360 volunteers and is in phase III (43,000 participants in the US, Argentina, Brazil and Germany). They evaluate doses of 60-100 µg of the vaccine candidate. For its part, Clover Biopharmaceuticals with a candidate expressed in CHO is studying doses of up to 30 µg of antigen in phase I. The candidate of Anhui Zhifei Longcom Biopharmaceutical consists of RBD expressed in CHO, like that of the Finlay Institute of Vaccines, Havana, and both are in phase II with doses of up to 50 µg of antigen in a three-administration schedule.

The COVID vaccines pharmacological and clinical studies on dose-response relationship in progress in the world consider the need to increase the dose of antigens.

Particularly, when proceeding to the evaluation in humans, and also considering the references of safety of the aforementioned vaccines, the maximum dose selected in the Abdala Study is 50 µg of RBD antigen; the distance between the administration of vaccine doses facilitates the maturation of the immune response, giving rise to the section of clones secreting antibodies, with greater avidity and a memory response.

However, short immunization schedules could also have practical utility by favoring short-term protective response necessary to control outbreaks and protection of personnel at immediate risk.

The increase in maturation and functionality of immune response also contributes to the administration of multiple doses. In the example of the vaccines against Hepatitis B, WHO recommends a diversity of schemes at **0, 1 and 6** months or **0, 1, 2, and 12** months. It is also accepted by regulatory authorities the scheme of **0, 7 and 21 days**. It is often demonstrate that a short scheme is inferior in term of guaranteeing long-term protection, but maintains its usefulness where emergency intervention is required.

7.4. Rules for the use of concomitant treatments

Volunteers have to know that, for the duration of the clinical trial, they should not receive other vaccines, experimental products, make blood donations, receive immunosuppressant or immunomodulator treatments in general, unless strictly necessary.

Individuals will be question about the habitual use of medications, as well as basic treatments, the details of which must be record in the Data Collection Notebook (DCN, in the section on concomitant therapy. Likewise, in the event that the need for concomitant medication arises, it will be analyzed, and indicate by the physician, and the details will be recorded in the DCN.

Any other treatment that the volunteer receives outside the context of the clinical site, it must be reported by the volunteer, and registered by the physician doctor in the DCN. The researcher must consider whether the medical condition that occurred and / or the required medical intervention should be consider for the exit of the study. He must also consider whether it is an adverse event.

If any type of allergic reaction occurs, it will be treat with the administration of antihistamines and steroids, as appropriate (at the discretion of the physician). The conduct before any adverse event will be the decision of the physician and will depend on the type, magnitude, and severity of the clinical manifestations in each case.

7.5. Measures to guarantee the safety in the handling of the product

The preparation and administration of the product will be carry out by personnel trained for these purposes, under the indication and supervision of the researcher. The product (CIGB-66 or placebo) will be stored at 2-8^{o}C (do not freeze) either in the promoter center, during transportation or while it is stored at the research site.

The CIGB-66 or placebo vials will be send to the Pharmacy of the Provincial Hospital "Saturnino Lora" in cases (individualized for each person). These kits will contain three kits (small kits) that correspond to the research product (CIGB-66 or placebo) that the included person should receive according to a randomized double-blind study).

Each kit will include:

- ✓ One vial of the product under study (CIGB-66 [25 or 75 µg] or placebo)
- ✓ One disposable syringe (1 mL)
- ✓ One needle 23G x 25 mm (for innoculation)

On the days provide in the trial schedule for the immunization of the participants according to the vaccination scheme to which they were randomly assigned, the person in charge of the Pharmacy will be responsible for the transfer of the product to the area of the hospital for the clinical trial

(vaccination). Only the dose (a kit) to be administer that day in the person will be extract from the Pharmacy.

The intra-hospital cold chain (2-8ºC) will be guarantee at all times. The refrigerator where the research product is store must has a temperature record. These temperature conditions will also be take into account during their transportation to the hospital, which will be the responsibility of the CIGB.

At the end of the study, the promoting center will proceed with the collection of the empty vials (and those not dispensed, if applicable), which will be destroyed at the CIGB according to the established procedures.

As it is a double-blind study, there are rules to be follow to guarantee adequate masking of the research product. In this sense, the label of the kit, for phase II, will specify:

1. Identification code of person (eg SL-159),
2. Name of two research products (CIGB-66 or placebo),
3. Expiration date (expiration date of research product that expires first),
4. Route of administration (intramuscular),
5. Storage temperature (2-8oC; do not freeze), and
6. Phrases “FOR CLINICAL TRIAL” and “SUPPLIED BY CIGB”,
7. Short name of the study and its code (ABDALA, IG / CIGB-66I / CVD19 / 2002),
8. Name of the hospital (Clinical Site)

BATCH is not included so as not to reveal the product

Case label example

FOR USE IN CLINICAL TRIAL

ABDALA Study (IG/CIGB-66I/CVD19/2002)

Clinical Trial Center: “Saturnino Lora” Hospital, Santiago de Cuba

Product: CIGB-66 (RBD 25 µg or 50 µg) or Placebo *Route: For Intramuscular (IM) Use*

Expiration Date: 03/2021 KEEP AT 2 a 8 °C. DO NOT FREZZE

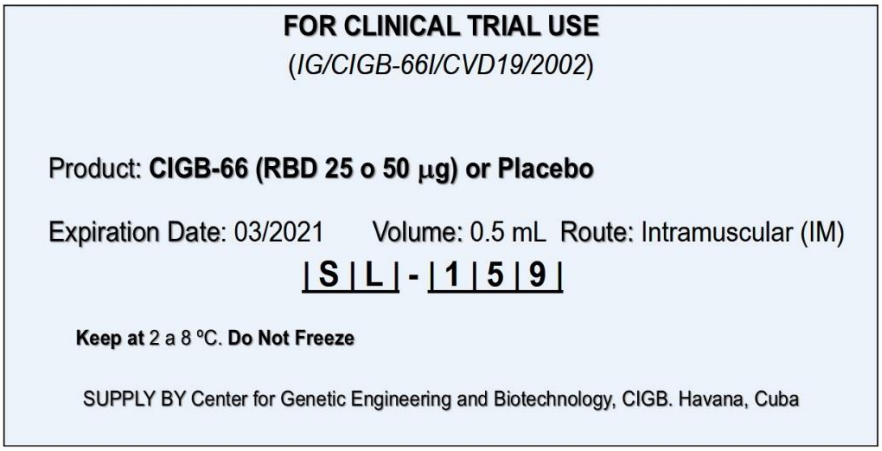
Participant TRIAL CODE : S L - 1 5 9

SUPPLY BY *Center for Genetic Engineering and Biotechnology, CIGB, Havana, Cuba*

The label of the vial (CIGB-66 or placebo), will specify the following aspects:

1. identification code of the person (ex. SL-159),
2. name of product (CIGB-66 or placebo),
3. expiration date (expiration of product that expires first) ,
4. volume (0.5 mL),
5. route of administration (intramuscular), t
6. he storage temperature (2-8oC; do not freeze), and
7. phrases “For clinical trial” and “Supplied by CIGB” and
8. Code of study (IG / CIGB-66I / CVD19 / 2002)

CIGB-66 or placebo vial label example:



Once administer the product and, the empty vials should be store, and refrigerated (2-80C).

These products must be stored, in their original case (supplied by CIGB), in a refrigerator designed for this purpose. The Head of the Pharmacy (or the designated specialist) will be responsible for the reception, storage, conservation and dispensing of the research product destined for the clinical trial; it must have a control of the entries and exits (cards and records) of the same. In the quality monitoring visits will be check, the dispensing records, the dispensed product (empty vials), as well as the temperature control records.

In case of accidental damage to a vial or if are detect foreign particles and / or color change (it must be transparent), **the ENTIRE vial is discarded**, the event is recorded and the main monitor is urgently notified for its timely replacement.

7.6 Measures to promote and control compliance with the instructions

The measures to ensure compliance with the prescribed instructions are specify below:

- The logistics to follow in the clinical site will be established in detail / explicitly to guarantee the process of selecting participants in the trial, obtaining informed consent (written), and the time of administration of the firtst dose of the research product (CIGB- 66 or placebo according to the randomized list), so that the research team achieves full adherence to the GCP and the test protocol.
- Each person will be evaluate throughout the study by the same medical team that included it, who will be in charge of filling out the DCN (Annex 13) and all the documents generated in the research. Personnel not linked to the study will not be allow to attend the cases or access the clinical trial product.
- Deliveries of the product, duly identified, will be make by the promoting center directly to the clinical site, which will be record in a control model for their distribution.
- The use of the product under study will be reserve only for the participants participating in the trial. All clinical researchers will have access to the product, which will be duly stored in the Hospital Pharmacy.
- Given the characteristics of the trial, where a significant number of volunteers will be innoculated on the same day (as projected), the *Supply group of the Direction of Clinical Research* of the CIGB will establish the requirements for the massive extraction of the product under study from the Hospital Pharmacy.
- The product is extracted by single medical prescription issued by the Main Researcher, on the days provide for the innoculation of the volunteers, with all the rigorous information for the extraction of the product, with the particularity that, in that prescription, it will request the extraction of the kit of all participants to be vaccinated that day; example: SL-001 - SL-066).
- The Pharmacy will only dispense the product to be administer on the same day of Main Researcher prescription date.

- Compliance with each innoculation of the product under study (date and time of administration) will be recorded in the DCN (Annex 13).
- The quality certificates of the research products (CIGB-66 and placebo) will be delivered to the clinical site before use. It must be kept in the Pharmacist's Folder.
- Researchers will fill out a registry of participants included (indicating the inclusion number) and not included (indicating the cause). In this model, only the initials of the person should be reflected as a contribution to the confidentiality of the data (Annex 7). This model will be sent to the CIGB at the end of the clinical trial; a copy of this record will be generated (self-replicating sheet) and kept in the researcher's folder.
- The main clinical researcher will have an internal registry of the participants included where they can fully reflect their personal data. This model will allow the location of individuals in any situation including absences to planned consultations (Annex 8). It will be kept at the clinical site and no copy will be generated for the center promoting the study.
- Clinical investigators will participate in an Initiation Workshop in order to study and achieve understanding / adherence to the approved protocol, and will be trained in GCP.
- The laboratory determinations provide for in the protocol will be carry out by personnel with experience in the different techniques and using validated methodologies.
- Empty vials after being used and those that are not used will be returned to the promoter center, for destruction according to the procedures established in the CIGB.
- During the progress of the investigation, quality monitoring visits will be carry 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. Likewise, the clinical site will be person to state inspections when decided by the national drug regulatory agency (Center for State Control of the Quality of Drugs and Medical Devices - CECMED).

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

7.7. Causes of treatment interruption

The causes of interruption and that could affect the administration stage of the research product are:

1) Voluntary abandonment.

2) Serious adverse event (with a proven causal link attributable to the research product).
3) Hypersensitivity reaction to the administration of the product.
4) Repeated adverse events with moderate intensity intolerable for the individual.
5) Appearance of any exclusion criteria detect after inclusion.

Note: Moderate adverse events may result in definitive discontinuation if the same event is repeat in the next administration of the research product unless, exceptionally, the physician decides to maintain the administrations considering the risk-benefit balance, and the consent of the individual.

Volunteers who receive at least one dose of the research product will be take into account throughout the follow-up to carry out the analyzes provide for in the protocol. Volunteers who have not received any doses will not be take into account for the analyzes.

Voluntary abandonment due to "loss of follow-up" will be consider participants who repeatedly absent themselves from scheduled visits in the trial and with whom the clinical site cannot establish contact.

VIII. EVALUATION VARIABLES

8.1. Protocol variables

8.1.1. Main variables

- **Security**

Clinical adverse events: The type, duration, intensity, causal relationship, conduct followed and outcome will be described (see section IX on adverse events). For this, in each dose the person will be evaluated through anamnesis, physical examination, vital signs and inspection at the inoculation site: before the administration of each dose of the product, in the first hour after inoculation of the product, at 24, 48 and 72 hours, and on the 7th day (all in person at the clinical site during phase I, although during phase II a face-to-face consultation will not be necessary on the 7th day) but also at any other time when an adverse event occurs.

As part of the safety profile, all participants will undergo hematological determinations (hemoglobin, hematocrit, hemogram with differential, platelet count) and blood chemistry (glycemia, cholesterol, creatinine, uric acid, glutamic pyruvic transaminase - TGP, glutamic transaminase oxaloacetic - TGO) at the beginning (time 0, before the administration of the 1st dose of the research product) and 14 days after the last dose of the product (day 42 or 70, according to the short or long schedule, respectively).

Additionally, each individual will have an "Adverse Events Card" where they can record any sign or symptom that occurs outside the healthcare unit, in addition to showing the doctor for evaluation. This model will not constitute an obligation to fill out by the participants.

- **Proportion of participants with SARS-CoV-2 anti-RBD IgG antibody seroconversion**

Seroconversion will be considered as that ≥ 4 times the initial determination of the antibody titer, on **days 42 and 56** (for the short scheme) and **56 and 70** (for the long scheme), with respect to the baseline time. During phase II, the assessment will be dispensed with on day 28.

Rapid recruitment of volunteers is anticipated as a result of the call for the ABDALA study. In the 1st phase, the necessary logistics will be organized to include the 132 participants required on two

different days (66 individuals per day). Likewise, for the second stage, recruitment will be organized in blocks until the sample size is completed.

Biological samples (serum) will be obtain under optimal biosafety conditions at the clinical site and will be frozen at -20oC until they are transferred to the CIGB where the planned immunological evaluations will be made.

The promoting center will be in charge of transmitting, operationally, the procedure for the extraction, identification and conservation of the biological samples that will be transferred to the CIGB to fulfill the planned objectives, according to the procedures 4.40.121.01 and 4.40.122.01 in force in the Investigations Direction CIGB Clinics. 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 healthcare unit will be the responsibility of the promoting center (CIGB), which will guarantee the transport, specialized personnel and resources necessary to carry out these operations with the maximum quality and compliance with the GCP, as established by the current procedures (4.40.120.01 and 4.40.123.07) and biosafety protocols.

8.1.2. Secondary variables

Geometric mean of anti-RBD specific IgG antibody titers on days **0, 42 and 56** (for the short vaccination scheme) and **0, 56 and 70** (for the long scheme).

Inhibition of the interaction of RBD with its ACE2 receptor by ELISA on days **0, 42 and 56** (for the short vaccination schedule) and **0, 56 and 70** (for the long schedule).

Additionally, other immunological tests may be explored that will not limit compliance with the objectives set forth in the research protocol. They are:

SARS-CoV-2 viral neutralization response percentage on days **0, 42 and 56** (for the short vaccination schedule) and **0, 56 and 70** (for the long schedule). In this case, only those samples that have ≥ 50% inhibition at 1/100 dilution in the surrogate RBD-ACE2 binding neutralization assay will be evaluate.

During phase II, the evaluation on day 28 will be dispensed, that is, **the secondary variables will be evaluated for the short scheme on days 0, 42 and 56**.

Evaluation	Frequency	Type / quantity of sample	device and Extraction volume	Analytical technique	Analyzing laboratory
Main variable					
Seroconversion of specific anti-RBD IgG antibodies (that ≥ 4 times the initial determination of the antibody titer)	28-42-56 (Short scheme) 28-56-70 (Long scheme))	Serum / 1 mL	Vacuum tube with separator gel to obtain serum (5 mL)	ELISA	ELISA Analytical Laboratory (CIGB)
Secondary variables					
Geometric mean of anti-RBD specific IgG antibody titers 0-28-42-56 (short diagram 0-28-56-70 (long scheme) Serum / 1 mL Vacuum tube with separator gel to obtain serum (5 mL) ELISA Analytical Laboratory (CIGB)	0-28-42-56 (Short Scheme) 0-28-56-70 (long scheme)	Suero / 1 mL	Vacuum tube with separator gel to obtain serum (5 mL)	ELISA	Analytical Laboratory (CIGB))
Surrogate Binding Neutralization Assay of RBD to ACE2	0-28-42-56 (Short Scheme) 0-28-56-70 (long scheme			ELISA	Analytical Laboratory (CIGB)
Exploratory tests					
SARS-CoV-2 viral neutralization response Only those with ≥ 50% inhibition at 1/100 dilution will be evaluated in the surrogate RBD binding neutralization assay to	0-28-42-56 (short scheme) 0-28-56-70 (long scheme	Serum / 1 mL	Vacuum tube with separator gel to obtain serum (5 mL)	viral Neutralization	Laboratory LISIDA

Biological samples destined to CIGB:

The process of obtaining serum from the blood samples taken will be carry out in the clinical laboratory of the hospital that runs the test, according to the routine procedures established for its blood chemistry determinations. The aliquots of serum destined for the CIGB will be stored, properly in the hospital at -20oC, until they are collected and transferred to the center promoting the study.

Sample processing:

- To obtain SERUM (a suggested procedure, although there are other methods):
- Incubate blood samples (without anticoagulant) for serum collection at 37 ° C for one hour.
- Subsequently, incubate at 4 ° C for 30 minutes.
- Centrifuge at 3000 rpm for 15 minutes.
- Extract the serum without breaking the clot. Transfer it to a plastic freezer vial. Identify the vial as appropriate. Discard the clot.
- Store the serum at -20ºC until analysis.

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

- With the intention of keeping the laboratory analysts responsible for immunological evaluations blind, each biological sample (serum) will be identified with a label that, in addition to specifying the short name of the clinical trial (ABDALA Study) and the type of biological material. (serum), will have a five-digit code, which will be unique for the person and unrepeatable, not being related to the code of the sample of that individual in another extraction time.
- The cryotubes where the samples will be collect will be previously labeled with the labels and packed in a plastic bag. To guarantee the control of these codes, a "Registry of Biological Samples" includes the number of volunteers and the box to register the code corresponding to the sample for each individual at each time.
- Each set of tubes with their labels will be randomly assigned by the person responsible for handling samples at the clinical site, who will keep that information for the laboratories that analyze the blind samples

- The cases or boxes for biological samples where the cryotubes will be placed (which will be transferred to the CIGB) will bear the code of the assay in their identification (IG / CIGB-66I / CVD19 / 2002; Study ABDALA) and the words “Biological Samples for the CIGB”.

Sample label for each serum sample:

It could be that the serum samples from person 001 at the initial extraction times and at 28 days are coded as follows:

17944	**14218**
SERUM	**SERUM**
ABDALA Study	ABDALA Study

Example of label of each box or cases where the samples will be kept (for the CIGB):

IG/CIGB-66I/CVD19/2002

ABDALA Study

Biological Samples to CIGB

Note: The hematological and blood chemistry determinations that will be carry out in the hospital's clinical laboratory will be record in the workbook corresponding to the clinical site, according to the usual established procedures. They will NOT be record in the model generated for the purposes of the clinical trial, reserved ONLY for the evaluations to be carry out at the CIGB.

The main clinical investigator will supervise the activity and the correct filling of the "Registry of Biological Samples". The Laboratory involved in the trial will be responsible for compliance with the activity, adherence to the protocol and the proper conservation of biological samples (until delivery to the promoting center).

Preservation of samples in the hospital:

1. The samples will be keep in a freezer at –20ºC until they are transferred to the CIGB.
2. The samples must be well stoppered and labeled with the specifications detailed above.

8.1.3. Control variables

- Age (years)
- Skin color (white, mestizo, black, yellow)
- Sex (male / female)
- Body mass index (Kg / m2)
- Toxic habits (smoking, alcohol consumption)
- Personal pathological history / risk factors (Yes, No, Type)
- Vaccination schedule (0-14-28 or 0-28-56) and dose level of the CIGB-66 vaccine candidate (25 or 50 µg).
- Concomitant treatments to the research product

8.2. Criteria to measure the response

Safety Criteria

- ✓ The product will be consider safe and well tolerated in a person when it is not associated with grade ≥ 3 adverse events according to CTCAE (Common Terminology Criteria for Adverse Events, v5.0: November 27, 2017).

Criteria for evaluating specific immune response

- ✓ Seroconversion of SARS-CoV-2 anti-RBD IgG antibodies estimated by ELISA (considering seroconversion as ≥ 4 times the initial determination of the antibody titer).
- ✓ Geometric mean of anti-RBD specific IgG antibody titers, Titers are logarithmically transformed and averaged, then compared between vaccination groups and at different evaluation times.
- ✓ Serum ability to block the interaction between the RBD protein and its soluble ACE2 receptor, evaluated by ELISA; To do this, the percentage of inhibition of the binding between RBD and its receptor ACE2 produced in a serum of a vaccinated person is calculated by means of a formula.

8.3. Periodicity of evaluations

The periodicity of the evaluations is shown below:

Evaluations Periodicity

SARS-CoV-2 by RT-PCR or other diagnostic procedure Pre-inclusion

Pregnancy test (quick strip supplied by the CIGB) Before each dose of the product

Clinical laboratory (hematology and blood chemistry) 0-42 (short scheme) 0-70 (Long scheme)

Seroconversion of specific anti-RBD IgG antibodies 0-42-56 (Short diagram) 0-56-70 (Long scheme)

Phase II:

0--42-56 (Short outline)

Geometric mean of anti-RBD titer

Surrogate Binding Neutralization Assay of RBD to ACE2

SARS-CoV-2 viral neutralization response *

* Only those with ≥ 50% inhibition at 1/100 dilution in the surrogate RBD-ACE2 binding neutralization assay will be evaluated.

8.4. Study success and failure criteria

- Individual success will be considered if the person does not interrupt the vaccination due to an adverse event, if no serious adverse events occur with proven causality, and if SARS-CoV-2 anti-RBD IgG antibodies seroconversion is achieved.
- The treatment will be considered successful if the hypothesis is proven.
- The definitive interruption of vaccination due to the appearance of serious adverse events with a causal relationship attributable to the research product or if seroconversion of anti-RBD IgG antibodies to SARS-CoV-2 is not achieved.
- Treatment failure will be considered if the study hypothesis is not proven.

IX. ADVERSE EVENTS

9.1, Adverse events that may occur and methods to record them

Adverse events will be classified according to type, duration, intensity, causal relationship, conduct followed and outcome, in the following grades55:

Grade 1 (Mild) Asymptomatic or symptoms of mild intensity, Only clinical or diagnostic observation. They do not require treatment.

Grade 2 (Moderate) Requires minimal, local or non-invasive intervention.

Grade 3 (Severe) Does not immediately compromise the life of the patient, Requires hospitalization (or if it is extended), Incapacitating.

Grade 4 (Severe) Endangers the patient's life, Requires urgent intervention.

Grade 5 (Serious) Death related to the adverse event.

The analysis of the causal relationship between the adverse event and the drug under study will be carry out using the following qualitative analysis:

1. Definitive: An event that
   1.1. shows a reasonable temporal relationship;
   1.2. follows a known response to the test drug;
   1.3. there is no reasonable explanation for its being caused by other factors such as the clinical condition of the individual or concomitant drugs administer;
   1.4. disappears when its administration is stopped and reappears when exposure is restarted.

2. Likely: An event that
   2.1. shows a reasonable temporal relationship after drug administration;
   2.2. shows a known response pattern to the test drug;
   2.3. cannot be explained by other factors such as the clinical status of the individual or concomitant drugs administer;
   2.4. disappears when its administration is stopped, but it is not confirmed with re-exposure.
3. Possible: An event that

3.1. shows a reasonable temporal relationship;

3.2. may or may not follow a known response pattern to the test drug; but that

3.3. may be caused by other factors such as the clinical condition of the individual or concomitant drugs administer.

4. Doubtful: The event is more likely related to other factors than to the drug involved.

Although it is a novel product, the RBD proteins obtain from different expression models have been evaluated in a large number of volunteers, with an adequate safety profile, Also, the adjuvant used has been widely used in humans, The main adverse events that could occur are fever, headache, nausea, pain / burning at the injection site, induration, erythema, general malaise, among others.

9.2, Procedure to follow in the face of adverse events

In this study, the occurrence of serious adverse events is not expected with the intramuscular administration of the vaccine candidate CIGB-66, which cannot be controlled without major consequences, In this clinical trial (ABDALA), in the event of an adverse event, it will progressively monitor and collect all the information concerning such incident (description of signs and symptoms, duration, behavior to mitigate it, etc.), The doctor must evaluate the causal relationship between each adverse event and the product under study (according to the criteria described in section 9.1), If any type of allergic reaction occurs, these can be controlled with the administration of antihistamines and steroids, as appropriate, In the event of serious adverse events, the vaccination will be suspended, the required measures will be take depending on the type of event and the IDMC will delve into the reported event and propose to the Promoter the conduct to follow.

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

9.3, Expedited reporting of adverse events

When a serious and unexpected adverse event occurs, the clinical investigator will notify (within the first 12 hours) the Clinical Trial Promoter (CIGB) and the CER / ECSR, The information may be communicated by phone, email or in person, The promoter will guarantee timely notification to the Cuban health authority (CECMED) in the following terms:

1. In the event of a serious and unexpected adverse event that causes the death or endangers the life of the person, it is reported as soon as possible from the moment in which the occurrence of the reaction is known.
2. In the event of a serious and unexpected adverse event that is not fatal or that is not life threatening, it will be reported as soon as possible and never after 15 calendar days from the first moment in which the occurrence of the reaction is known. .

The hospital will complete the Report of Unexpected Serious Adverse Events, issued by CECMED in its Regulation 45-2007 (Requirements for the notification and reporting of serious and unexpected adverse events in clinical trials), This model (general in any clinical trial carry out in Cuba) will be available.

It is the responsibility of the clinical investigator to inform the study monitor of any serious adverse event that occurs and to determine the measures to be take in each case to protect the study participants.

CIGB monitor to contact:

Dr.C, Francisco Hernández Bernal (72080428 / 72087465 (ext. 148).

Corporate mobile line: 52168101 *✉hernandez.bernal@cigb.edu.cu

X. DATA COLLECTION AND HANDLING

10.1. Information collection model

FORM (Annexes)	Time to filled out Study Information	Information to collect	Responsible
Consent / Information to the person (**Annex 4 and 5 / 5a**)	Before inclusion,	Voluntary confirmation, in writing, of the person participating in the trial, Synthesized information about the trial in question, its objectives, possible benefits, rights, risks and disadvantages,	Clinical researcher
Commitment of the Principal Clinical Investigator (**Annex 6**)	Before the start of the study	Commitment to compliance with GCP and adherence to the protocol by the researchers	Principal investigator
Registry of included and not included participants (**Annex 7**)	Period of inclusion of cases,	List of all participants who responded to the trial call (included or not and the causes),	Clinical researcher
Investigator Record (**Annex 8**)	When the person is included in the study,	Identification data of the participants, with general ones for their quick location,	Clinical researcher
Registration of authorized signatures in the clinical trial (**Annex 9**)	Before the start of the study,	Control of signatures of researchers authorized to complete the primary information generated in the study (at the clinical site and at the CIGB),	Monitor
Adverse Events Card (**Annex 10**)	At the time the information is generated	Record (by the person participating in the study) of the adverse events that occur while on an outpatient basis,	Clinical researcher
PRE-inclusion check (**Annex 12**)	Before inclusion,	Pre-inclusion check (planned during the 1st stage of the study, where the selection criteria are verify.	Clinical researcher
Data Collection Notebook, DCN (**Annex 13**)	At the time the information is generated	General data, compliance with treatment, laboratory and evaluations, adverse events, other issues related	Clinical researcher

Copies of Models listed below will be obtain by the "*not copy required*" system (self-replicating sheet), which allows the immediate generation of copies of the original document after fill out:

- Informed Consent (**Annex 4**)
- Registry of participants included and not included (**Annex 7**)
- Data Collection Notebook - DCN - (**Annex 13**)

In the case of the Informed Consent model (**Annex 4**), the responsible researcher will keep the original document (the established time of 15 years, as part of the primary information of the clinical

trial), and will provide the person participating in the study with a copy of the document itself (together with the information sheet - **Annex 5**).

In phase II, the information sheet is adapted to the person by decision of the Promoter in the intermediate analysis (**see Annex 5.a**).

In the case of the registry of *included and not included participants* (**Annex 7**) as well as the DCN (**Annex 13**), the researcher will retain the copy of the specimen and send the original to the CIGB for data management and statistical analysis, in addition to its adequate conservation for 15 years.

The researchers will complete the information requested in the DCN as it is generate, after the last evaluation (or during interim reviews), the DCN (Annex 13) is sent 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 is blank, not filled.

Concerning the questions where the information cannot be obtain, the procedure indicated in the "*Rules for filling out the DCN*" (See Instructions for filling out the DCN will be follow.

Annotations will be make preferably in black ink or blue as second option, and there will be no erasures or blots, or illegible letters or words; if a correction is necessary, the incorrect value or data will be crossed out with a single line and the correct result will be noted, it will never be erased.

10.2. Procedures for keeping information

The CRDs, the databases and the reports that are generated will be kept (printed and in optical format) in the Direction of Clinical Investigations of the CIGB (Documentation Group and BPC) for at least 15 years.

The primary documentation generated in the study, in the possession of the responsible researcher in each clinical site, must be keep for the same period (including the medical records that will be prepared in the participants included in the first stage; not in those that will be included in the next stage after the interim analysis).

The final report of the study will be made analyzing each of the results obtain in the statistical processing, This information is "restricted", therefore only the researchers who participate in the study will have access to it, and their care and conservation are their responsibility.

10.3, Data management and preservation

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 DCN design, which allows the capture, storage and electronic management of data,

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

The information will be enter independently by two operators in duplicate for the subsequent automatic comparison, and correction of the bases, necessary for the statistical analysis with the accurate information from the trial

For the debugging of errors, the data that does not agree with that registered in the DCN will be corroborate so as not to lead to confusion. This activity will be record, in a way that allows its traceability before national and foreign inspections and / or audits.

XI. STATISTICS

11.1. Number of planned participants

- PHASE I:

The calculation to complete the first phase of the study is make considering two intramuscular immunization schedules: 0-14-28 and 0-28-56.

With a sample size of 20 participants, the 95% confidence interval can be estimate for a proportion, assuming a rate of related serious adverse events of less than 5%.

Numeric Results for Two-Sided Confidence Intervals for One Proportion
Confidence Interval Formula: Exact (Clopper-Pearson)

			Sample				
Confidence Level	Size (N)	Target Width	Actual Width	Proportion (P)	Lower Limit	Upper Limit	Width if P = 0.5
0.950	20	0.250	0.247	0.050	0.001	0.249	0.456

To fulfill the hypothesis with a preset precision of 0.05, power of 80% and percentage of loss due to study exits of 10%, 22 participants per group would be need, Total sample size of phase I of **132 volunteers.**

If any of the treatments satisfies the stop criterion after the administration of the first or second dose, the immunization schedule will not be complete, and inclusion in that group will not be continue.

At the conclusion of the treatment regimens, the two best experimental groups are select based on the benefit / risk that will go to phase II of the clinical trial, to fulfill the hypothesis foreseen for that stage could be selected for the second stage). (Selection independent of the immunization regimen, that is, that an experimental group from each evaluated scheme or the two experimental groups from the same immunization scheme

- PHASE II:

The sample size is calculated to estimate a difference in proportions of approximately 50%, in such away that the lower limit of the confidence interval for the difference between treated, and control is greater than 30% (which is the criterion of success. adopted by other regulatory agencies for

vaccines intended for COVID-19). Assuming that in the control group the proportion of individuals with an immune response can range between 1-5%

Confidence Intervals for the Difference Between Two Proportions

Numeric Results for Two-Sided Confidence Intervals for the Difference in Proportions
Confidence Interval Method: Chi-Square - Simple Asymptotic (Pearson)

Confidence Level	N1	N2	N	Target R	Actual R	Target Width	Actual Width	P1	P2	P1 - P2	Lower Limit	Upper Limit
0.950	415	415	830	0.500	0.501	0.100	0.100	0.51	0.01	0.50	0.45	0.55
0.950	185	185	370	0.500	0.503	0.150	0.150	0.51	0.01	0.50	0.43	0.57
0.950	104	104	208	0.500	0.500	0.200	0.200	0.51	0.01	0.50	0.40	0.60
0.950	47	47	94	0.500	0.511	0.300	0.297	0.51	0.01	0.50	0.35	0.65
0.950	26	26	52	0.500	0.500	0.400	0.399	0.51	0.01	0.50	0.30	0.70
0.950	527	527	1054	0.500	0.501	0.100	0.100	0.55	0.05	0.50	0.45	0.55
0.950	220	220	440	0.500	0.500	0.150	0.150	0.55	0.05	0.50	0.43	0.57
0.950	132	132	264	0.500	0.500	0.200	0.200	0.55	0.05	0.50	0.40	0.60
0.950	59	59	118	0.500	0.508	0.300	0.298	0.55	0.05	0.50	0.35	0.65
0.950	33	33	66	0.500	0.515	0.400	0.398	0.55	0.05	0.50	0.30	0.70

Dropout-Inflated Sample Size

	Sample Size				Dropout-Inflated Enrollment Sample Size			Expected Number of Dropouts	
Dropout Rate	N1	N2	N	N1'	N2'	N'	D1	D2	D
10%	220	220	440	242	242	484	22	22	44

Considering a percentage of loss due to dropouts of 10%, 242 participants per group would be needed.

- If in the interim analysis an experimental group from each immunization scheme is selected for the second stage:

  88 participants who were included in stage I should be subtracted (44 treated and 44 controls; half in each scheme),

  Therefore, **a total of 880 individuals would be added in phase II: 220 treated and 220 controls** in each immunization scheme, A total of 1012 individuals (132 in phase I and 880 in phase II) would be included in the study.

- In the event that in the interim analysis the two experimental groups of the same immunization scheme are selected for the second stage:

  66 participants who were included in phase I (44 treated and 22 controls) would have to be subtracted, Therefore, **a total of 660 individuals would be added in phase II**: **440 treated** (220 at each dose level) and **220 controls**, A total of **792 individuals** (132 in phase I and 660 in phase II) would be included in the study.

11.2, Planned statistical analysis

11.2.1, Analyzed data set

The following populations are distinguished:

"***By protocol***": defined as the volunteers who have been included, who meet all the selection criteria, who have received the complete immunization schedule, in which the assessment of the main variable is available, and who have not suffered any major deviation of the protocol, Individuals will be considered in the group where they were randomized. All immunogenicity variables will be calculated in this population.

"*Intention to treat*": all volunteers who have received at least one immunization, In this population, all demographic and baseline variables will be studied, and of the response variables the proportion of participants with seroconversion of anti-RBD IgG antibodies to SARS-CoV-2, Individuals will be considered in the group where they were randomized.

"***Safety population***": Are all volunteers who have received at least one immunization, Individuals will be consider in the group where they were treated.

11.2.2. Exploratory analysis

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

Disruptions will be analyze through contingency tables with proportions and percentages, the Chi-square test, or Fisher's exact test will be used to evaluate independence with respect to treatment, and causes will be listed.

11.2.3, Confirmatory Analysis

- ✓ **In phase I:**

Primary variable: Security

- The percentage of individuals with serious or serious adverse events related to the research product will be estimate (during the period of execution of the clinical trial) and the 95% confidence interval will be calculate, In case the frequency is very low, the Bayesian confidence interval will be estimated.
- The frequency of individuals with each adverse event will be estimate.
- The frequency distributions of each type of reported event will be shown (if necessary, common entities will be recoded), of the intensity, duration, severity, result and causal relationship,

Laboratory Evaluations

- An ANOVA model with repeated measurements will be adjusted to evaluate the effects "*treatment*", "*time*" and "*interaction treatment time*", after verification of the assumptions for its validity: approximation of the data by a normal distribution (test Kolmogorov Smírnov), homogeneity of variances (Levene's test), compound symmetry (Mauchly's test of sphericity).
- , If this is not possible start, end paired analyzes will be perform in each group (Student's t test for dependent samples or Wilcoxon test), depending on the assumption of approximation of the data by a normal distribution.
- The start end difference in each group with respect to the control group will be evaluated using the Student's t test (after verification of the assumptions of normality and homogeneity of variances) or the Mann Whitney U test.
- Graphs will be present that show the evolution of the values of central tendency and dispersion.

Stop criterion due to unacceptable toxicity: It will be evaluated iteratively, if the probability that the proportion of individuals with related serious adverse events is higher than 5% is high (≥ 0.90), For this, the independent Data Monitoring Committee will accompany the evaluation of the study data to make appropriate recommendations to the promoter.

The evaluation will be perform under the following procedure:

• Assume non-informative a priori density function:

$$beta(u;a,b) = \frac{u^{a-1}(1-u)^{b-1}}{B(a,b)}, \qquad 0 < u < 1,\ B(a,b): \textit{función beta}$$

• Estimate the probability of toxicity according to Bayes' Theorem:

Prob (toxicity) = $Beta(a + X_{jm}; b + m - X_{jm})$,

Where:

Xjm: number of participants with a serious / serious adverse event and a causal relationship ?

If $P\left[toxicidad_j > 0.05 / X_{j,m}\right] = 1 - \int_0^{0.3} beta(u; a + X_{j,m}, b + m - X_{j,m})du > 0.90$ inclusion is stopped due to unacceptable toxicity

Prob (toxicidad)= $Beta(a + X_{jm}; b + m - X_{jm})$,

Where:

X_{jm}: number of persons with serious/severe adverse event and causal relationship

Si $P\left[toxicidad_j > 0.05 / X_{j,m}\right] = 1 - \int_0^{0.3} beta(u; a + X_{j,m}, b + m - X_{j,m})du > 0.90$

Inclusion halted by inadmissible toxicity

Criteria for selecting the best treatment:

It will be evaluate based on the *Risk-Benefit Balance*, considering two scenarios:

Scenario 1:

Benefit:

Greater proportion of participants at the evaluated times, with respect to baseline time, .with SARS-CoV-2 anti-RBD IgG antibodies seroconversion (considering seroconversion as ≥ 4 times the initial determination of the antibody titer).

Risk:

Occurrence of serious adverse events in the 28 days after the application of each dose with a proven causal relationship

Scenario # 2:

Benefit: Serum's ability to block the interaction between the RBD protein and its soluble ACE2 receptor, evaluated by ELISA (considering those with ≥ 50% inhibition at a 1/100 dilution in the surrogate RBD binding neutralization assay to ACE2)

Risk: Occurrence of serious adverse events with a proven causal relationship in the 28 days after the application of each dose

The following expression will be use:

$$\text{Bayes Factor} = B(x) = \frac{\pi(beneficio \mid x)/\ p(beneficio)}{\pi(riesgo \mid x)/\ p(riesgo)}$$

In addition, as a decision criterion:

If B (x) ≥ 1: Evidence in favor of the benefit.

If 1 > B (x) ≥ $10^{-½}$: Minimal evidence against the benefit.

If 10^{-1} / 2> B (x) ≥ 10-1: Substantial evidence against benefit.

If 10^{-1}> B (x) ≥ 10-2: Strong evidence against the benefit.

If 10^{-2}> B (x): Decisive evidence against benefit.

For decision making, viral neutralization responses and anti-RBD MGTs will also be considered, The state of the art will also be take into account in terms of possible findings in immune protection (benefit) or toxicity (risk) and finally practical factors such as the candidate that is acceptable in terms of safety and immune response, but that offers advantages. in its application or economic profitability

- ✓ In phase II:

Main effect variable: Proportion of participants with seroconversion of anti-RBD IgG antibodies to SARS-CoV-2 (considering seroconversion as ≥ 4 times the initial determination of the antibody titer), on the days evaluated, with respect to the base time

- Estimate for each age cohort and in total, the probability of success of each treatment group with respect to the control group, P (θA> θB + 0.5 / data) from the Bayesian point of view using the following expression:

$$\sum_{y_A,y_B} P(Y_A = y_A/X_A)\, P(Y_B = y_B/X_B)[P(\theta_A > \theta_B/datos)]$$

Where θA, θB probabilities of success of both treatments, respectively

X_A, X_B Number of successes observe in both treatments, respectively

Y_A, Y_B Number of predicted successes in both treatments, respectively

Non-informative *a priori* distributions will be assumed for the parameters θA and θB, in particular beta distributions [1,1].

- Estimate the confidence interval of the difference with respect to the control of the proportion of participants with seroconversion of anti-RBD IgG antibodies.

Secondary variables:

1. Percentage of seroconversion of specific anti-RBD IgG antibodies on days 0-28-42 (short panel) and 0-28-56-70 (long panel)

2. Humoral response - anti-RBD specific antibodies (IgM) on days 0-28-42 (short frame) and 0-28-56-70 (long frame)

3. Geometric Mean of the anti-RBD specific IgG antibody titer on days 0-28-42 (short plot) and 0-28-56-70 (long plot)

4. SARS-CoV-2 viral neutralization response percentage, on days 0-28-42 (short frame) and 0-28-56-70 (long frame)

5. Inhibition of the interaction of RBD with its ACE2 receptor by ELISA on days 0-28-42 (short scheme) and 0-28-56-70 (long scheme)

I. For quantitative response variables:

- Estimate the measures of central tendency and dispersion (mean, median, standard deviation, interquartile range, and minimum and maximum values) in each dose group.

- Estimate the confidence intervals of the difference with respect to the control.

- Compare the groups using ANOVA or its corresponding non-parametric alternative (Kruskall-Wallis test), depending on the fulfillment of the distributional assumptions.

II. For qualitative response variables:

- Perform univariate analyzes (frequency distributions, Chi-square statistic or Fisher's exact test) and multivariate (Logistic Regression Models) to determine the relationship between the control variables and the response variables.

11.3. Evaluation of extreme values and missing data

For the response variables (immunogenicity), box and whisker plots will be made to detect exterior values (outliers) and descriptive measures such as the interquartile range will be used.

The behavior to be followed in the face of the extreme value will be done jointly with the investigators-monitors, after verifying the quality of the data, comparing the results with and without the detect value, In the event that discrepancies are found between the two analyzes, it will be reported and discussed in the final statistical report.

Missing values in the main variables of safety and immunogenicity will be considered "Missing at random (MAR)" and therefore will be ignored in the primary analysis. T

hey will not be charged, However, if more than 5% of all responses for all variables included in the main analysis are reported as missing data, a sensitivity analysis will be carry out, which will include the results of the models under the following assumptions:

-Dragging the last observation (if possible)

-Imputation for worst-case scenario

11.4. Rules for ending the trial

The trial will be terminate when the last volunteer included ends the follow-up.

The following systems will be used for statistical analysis: SPSS version 25.0, STATISTICA version 6.1, R version 3.5.0, EPIDAT version 3.1 and WinBugs version 1.4.

XII. RESOURCES ASSURANCE

Resources	Responsible	Quantity
CIGB-66 / Placebo	CIGB	3100
Samples for immunological determinations	CIGB	4000
Samples for hemochemical determinations	Clinical Site	2000
Pregnancy test	CIGB	1500
Data Collection Notebooks, and other models by "*not copy required*" system	CIGB	1000
Research Protocol / Annexes (Reproduction)	CIGB	50
Laboratory material (vacutainer, reagents, various supplies)	CIGB	-
Other Hospital Supplies	Clinical Site	-
Logistics (workshops / meetings; quality monitoring visits; inspections; transportation),	CIGB -	

XIII, GENERAL CALENDAR.

Issue	Start	Completion
Coordination and preparation of the protocol	November 5, 2020	November 10, 2020
Approval of the Research Ethics Committee	November 16, 2020	November 20, 2020
Approval by CECMED	November 20-30, 2020	
Workshop and initial visit of the protocol	December 2, 2020	
Execution of the study	December 7, 2020	July 15, 2021
Processing and analysis of results	July 30, 2021	
Preparation of the final report,	August 31, 2021	

XIV, PRACTICAL CONSIDERATIONS

14.1. Distribution of duties and responsibilities in the protocol.

14.1.1, Sponsor 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. Appoint the Clinical Trial Monitor, who will be responsible for the study as a representative of the Sponsor's interests.
4. Carry out a workshop to unify criteria and start, where the protocol proposal is presented and discussed
5. Inform researchers about the chemical-pharmaceutical, pharmacological, toxicological and clinical properties of the product under study, However, this obligation does not mean 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 professional training.
6. Provide, during the clinical trial, any new information relevant to the conduct of the study that comes to your attention.
7. Retain primary information and all research data for 15 years.

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

1. Participate in the design and preparation of the Clinical Trial Protocol.
2. Present the protocol to the CER / ECSR of the hospital, always through the coordinating clinical investigator.
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. Register the study in the Cuban Clinical Trials Registry, WHO primary registry.
5. Request the CIGB Regulations Department to process it before CECMED for the authorization to start the clinical trial in Cuba.
6. Carry out quality monitoring visits during the execution of the study.

7. Immediately notify the Sponsor and CECMED of the appearance of any serious adverse event that occurs in the participants participating in the trial.
8. Verify the adherence of researchers to the approved protocol.
9. Inform the clinical site (and the CER / ECSR) about modifications to the protocol.
10. Participate in the preparation of the Final Report and the publication jointly with clinical researchers, product leaders, and study advisors.

14.1.3, Duties of the Principal Clinical Investigator

1. Prepare the research protocol together with the study monitor.
2. Send a copy of your Curriculum Vitae to the promoting center.
3. Participate in the quality monitoring visits carry out by the monitor; guarantee all documentation.
4. Deliver the protocol to CER / ECSR for approval, Inform the latter if important situations occur in 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 the intention to disclose the results, whether preliminary, partial or total, the authorization of the promoter must be obtain and all the considerations detailed in the point "*Considerations on confidentiality*" must be comply with.
7. Verify compliance with the responsibilities of the co-investigators.
8. Keep the documentation generated during the trial for 15 years.

14.1.4, Duties of Co-Investigators

1. Know all the necessary information about the product under study, as well as its possible adverse events and be prepared for their treatment in case of occurrence.
2. Participate in the criteria unification activities that are carry out.
3. Send a copy of your Curricula Vitae to the promoting center.
4. Keep the clinical trial documentation updated.
5. Ensure compliance with the protocol, ethical principles and GCP.
6. Include all participants that meet the selection criteria.
7. Obtain written informed consent from the participants before the start of the study.

8. Register the information of each person in the corresponding DCN.
9. Have available all the information that is requested in the quality controls on the site and in the check-up visits, Deliver the completed CRDs to ensure the speed and quality of the study data entry process.
10. Inform the Promoter (Monitor) and the CER / ECSR about any serious adverse event (first 24 hours).
11. Maintain the confidentiality of the information generated during the execution of the trial, In the event of the intention to disclose the results prior agreement with the principal investigator, whether preliminary, partial or total, the sponsor's authorization must be obtain.
12. Keep the documentation generated during the trial for 15 years.

14.1.5, Duties of the Hospital Management

1. Guarantee that the personnel who will participate in the investigation have the time necessary to carry out their tasks-time necessary for the fulfillment of the tasks of the same.
2. Have knowledge of the objectives of the test and the necessary requirements to guarantee its quality.
3. Support researchers in everything related to the collection of clinical information.
4. Ensure that no other investigation is being conducted in the same service that competes with this one.
5. Support the investigation, as well as the quality controls that are programmed.
6. Contribute to communication between the researcher and the promoter.

14.2, Procedures for the flow of documentation and medications

The research product, DCN and other models, will be delivered / collected by the CIGB, directly by the studio monitors, The monitors, in the quality monitoring visits, will collect the CRDs once the participation of the participants in the trial has concluded, The Supplies group of the CIGB Clinical Research Direction will be responsible for the adequate supply of vaccines (CIGB-66 or placebo) and medical supplies, as well as for the collection of the product under investigation (dispensed and not dispensed).

The investigator must have a report on the number of participants included, the adverse events detect, the study exits and their causes, as well as any other relevant information during the course of the trial, for when they are requested during the visits. quality monitoring, In Annex 11 you will find the location of the personnel linked to the research to establish any type of communication in relation to the clinical study.

14.3. Considerations on confidentiality, disclosure of results and other legal aspects.

The ownership of the results and data obtain in the clinical trial will correspond exclusively to the Study Sponsor (CIGB), who reserves the right to use them to submit them 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 obtain during the investigation, It also undertakes to respect the confidential nature of the results and data obtain during this clinical trial.

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

A Work Contract will be signed between the CIGB and the Provincial Hospital "Saturnino Lora" which documents the will of the parties to carry out the clinical trial and the rights and obligations of each will be established, with the aim of guaranteeing the fulfillment of activities during the study, Before signing the contract, the CIGB must have the opinion of approval of the study by MINSAP.

The promoter will not establish third-party agreements with the clinical researchers from which additional financial compensation or other types of consideration are derived, except for the expenses of meetings for the organization of the study, as well as those facilities that the promoter

may have in the future for dissemination. of the results obtain from the study in scientific meetings and publications.

The CER / ECSR of the clinical site participating in the study will be asked for the approval of the clinical trial protocol; its members will be listed in it, as well as the date and signature of each one, as proof of their approval for the execution of the test in the institution.

The official list of firms authorized in the study will be collected, made up of the researchers who will participate in the clinical trial, as well as by appointed and authorized support personnel (for example, clinical research coordinators), 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 will be accessible to Cuban and foreign auditors interested in the investigation.

The monitors designated by the sponsor may access the information and clinical documentation on the individuals included in the study, in order to verify the accuracy and reliability of the data provide by the clinical investigators, but they do not have to collect personal identification data. of the study participants, The clinical site will also facilitate access to these data, to the inspectors of the competent health authorities.

Before the start of the clinical trial, the Promoter will subscribe with the National Insurance Company of Cuba (ESEN), an insurance policy for civil liability for clinical trials, which covers the responsibilities of the Promoter, the researcher and their collaborators, in the terms provide by Law. The Promoter undertakes to maintain insurance coverage throughout the duration of the clinical trial.

14.4, Quality Assurance Plan

Monitoring will be carry out at regular intervals, at least every two weeks, depending on the progress of the investigation, The objective of the control visits is to verify adequate compliance with the GCP / adherence to the protocol and thus verify the efficient execution of the investigation, Likewise, these visits will serve to discuss any aspect of the protocol that the researcher suggests, They will be carry out by the promoter center monitors.

Each visit will be announced in advance, The researcher and the hospital unit to which he belongs will allow the CIGB monitors to inspect and review the trial documents, including the CRDs and the use of the resources provide, The principal clinical investigator should have all study documentation ready and available for review, The aspects to be monitored and controlled are: compliance with the provisions of the protocol, registration of included and not included, storage conditions and use of the product under study, destination of the resources provide, fidelity of data collection and safety profile (adverse events).

In anytime of the trial researchers, product leaders and study advisors could perform inspections on behalf on the Official state authority (CECMED).

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