Re: 20-36255 – Efficacy and Safety of Ivermectin for Adult Patients with Mild Covid-19

This supplement contains the following items:

1. Original Protocol

2. Final Protocol

3. Summary of Changes in the Protocol

4. Original Statistical Plan

5. Final Statistical Plan

6. Summary of Changes in the Statistical Plan

DOUBLE BLIND, RANDOMIZED, CONTROLLED CLINICAL TRIAL TO INVESTIGATE THE EFFECTIVENESS OF THE D11AX22 MOLECULE IN ADULT SUBJECTS FROM CALI WITH INITIAL STAGES OF INFECTION BY SARS COV2 / COVID-19

Protocol Date: Final Version 2.0: May 12, 2020

Protocol Amendment Date: Final Amendment 1: June 9, 2020

PRINCIPAL INVESTIGATOR

Eduardo López Medina, MD MSc
Center for Studies in Pediatric Infectology
Universidad del Valle
Imbanaco Medical Center

DIRECTION AND CONDUCT OF THE TRIAL

Centro de Estudios en Infectología Pediátrica --CEIP-- S.A.S

TRIAL SPONSOR

Centro de Estudios en Infectología Pediátrica --CEIP-- S.A.S
Calle 5 B 5 No. 37Bis - 20
Cali Valle
COLOMBIA

Investigator Agreement to Protocol

I agree with:

- Conducting the study in compliance with this protocol, any future modifications to the protocol or mutually agreed administrative changes to the protocol, the terms of the study agreement, and any other study development procedures and/or study development documents.
- Assuming responsibility for the proper conduct of the study on this site.
- Being aware of and comply with the "Good Clinical Practices" (GCP) and all applicable regulatory requirements.
- Ensuring that all persons assisting me with the study are adequately informed regarding the duties and functions related to the study as described in the protocol.
- Acquiring reference ranges for laboratory analyzes performed locally and, if required by local regulations, obtain current laboratory certification or the Quality Assurance Procedure Manual.
- Ensuring that no samples (including serum samples) are retained at the site or elsewhere without the express written informed consent of the research participant.
- Not carrying out other biological tests on the samples, except those described in the protocol or its modification (s).

1. INTRODUCTION

Since the discovery of human coronaviruses (HCoV) in the 1960s, 6 viruses, including HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV (severe acute respiratory syndrome) and MERS-CoV (Middle East respiratory syndrome), have been recognized as agents causing respiratory infections, some causing severe life-threatening multisystemic syndromes. HCoV NL63 and HCoV HKU1 were described in 2004 and 2005, respectively, and together with HCoV 229E and OC43 are responsible for up to 35% of upper respiratory infections, generally in epidemic outbreaks.[1]

HCoV OC43 is the most prevalent, affecting mostly children under 5 years of age. It is very frequently identified in coinfections with other respiratory viruses, which makes it difficult to determine its true role. In addition, these have been associated with more serious conditions, which lead to hospitalization and even fatal cases. Several members of the Coronaviridae family are constantly circulating in the human population and generally cause mild respiratory illness.[1]

The most severe disease-causing coronaviruses in humans are SARS-CoV and MERS-CoV, both of which are transmitted from animals to humans and cause severe respiratory illness.[2,3]

At the end of 2019, a series of cases of severe acute respiratory infections with characteristics that had not been previously described were identified in China. Later it was shown that these characteristics were produced by a new coronavirus that was called SARS-CoV-2, which causes COVID-19.[4] The virus spread rapidly throughout the world, causing more than 2,200,000 cases by April 25, 2020, with nearly 100,000 deaths.[5]

It is a single-stranded RNA virus, which makes it easy to mutate, rapidly adapting to new hosts. It belongs to the Beta coronavirus family and can infect type 2 pneumocytes and ciliated bronchial epithelial cells producing an acute respiratory disease whose most common symptoms are fever, cough, nausea or vomiting and diarrhea, with varying degrees of severity, producing severe illness in older subjects and those with associated comorbidities such as high blood pressure, heart problems or diabetes.[6] About 80% of people recover from the disease without the need for any specific treatment, however, 1 in 5 people who contract the virus progress to severe illness.[7]

The diagnosis is made by identifying the genetic material of the virus by real-time polymerase chain reaction (RT-PCR) of the affected airways. Among the limitations of this test is that the SARS-CoV2 infection can only be determined at the time of the test. We cannot know if the subject was infected days before it. Furthermore, although RT-PCR is a relatively reliable technique, the sensitivity depends on the way the sample to be processed is obtained.[8]

Because it is a new virus, we do not have previous immunity to it, which makes us susceptible to infection. We also do not have antiviral drugs or effective vaccines that can mitigate the infection.[9] Currently, there are hundreds of clinical studies looking for the best therapeutic strategies, but no randomized clinical study has yet demonstrated effective therapeutic strategies in the early stages of infection. Given the high morbidity and mortality that this disease generates in its most advanced stages, the global urgency to find measures that reduce the impact of this disease and the delay that represents the generation of new

antiviral drugs, it is essential to evaluate in a clinical trial whether already available drugs that have demonstrated safety and benefit in observational or in-vitro studies against SARS-CoV-2, maintain their usefulness in early stages of COVID-19.

2. PROBLEM STATEMENT AND JUSTIFICATION

Valle del Cauca is the department with the second highest number of cases and lethality in Colombia. As of April 26, 2020, we have 841 cases, of which 12% have required hospitalization and 8.7% require intensive care, with a fatality rate of 6.5%.[10] Every day a greater number of cases are reported in Valle del Cauca.

In the early or mild phases of the infection, the currently recommended procedure is symptom control, with no medication proven to reduce disease progression.[11] However, about 20% of subjects progress to severe disease, so it is urgent to identify interventions that mitigate this progression. This is especially important considering that, as with other antiviral medications, early reduction in viral load during mild forms of the disease has favorable effects on its outcome.[12]

The D11AX22 molecule (ivermectin) is a new therapeutic proposal against COVID-19. The D11AX22 molecule (ivermectin) is one of the most important drugs ever discovered and has been recognized as a benchmark by the American Chemical Society. Its discoverers were awarded the Nobel Prize in 2015 for demonstrating its great efficacy as a broad-spectrum antiparasitic.[13] Originally introduced as an antiparasitic in veterinary medicine in the early 1980s, it is a drug that eliminates a wide range of endo and ectoparasites in livestock and other animals. For more than 35 years it has been used in humans with great efficacy and safety to treat endoparasites that are difficult to control, such as filariasis, onchocerciasis or strongyloidiasis, also ectoparasites, such as pediculosis capitis and myiasis.[14-16]

Its antiviral effect has recently been evaluated against SARS-CoV-2 and other viruses. The IMP heterodimer$\alpha\beta$ 1 is a molecule that binds to the viral load protein and transports it to the nucleus, reducing the antiviral capacity of the host cell. The D11AX22 (Ivermectin) molecule destabilizes this heterodimer, prevents its binding to the viral protein, and prevents its entry into the host cell nucleus.[17] Other actions of the D11AX22 (ivermectin) molecule include the ability to inhibit nuclear importation of integrase and HIV viral replication,[18] inhibition of dengue virus NS3 helicase (DENV)[19]; and it has been shown to be a potent inhibitor of the yellow fever virus replication through its action on the viral helicase, preventing the synthesis of viral RNA.[19] The D11AX22 (ivermectin) molecule has also been shown to inhibit the importation of different proteins into the nucleus, including tumor antigen (T-ag) from simian virus SV40 and DENV NS5, and limits infection by other viruses, including DENV serotypes 1-4 and influenza[18,20 ,21 ,22,23] due to the dependence of RNA virus on IMP$\alpha\beta$ 1 during infection.[24,25] The D11AX22 molecule (Ivermectin) was evaluated as a treatment in humans with dengue in a phase 3, randomized, double-blind, placebo-controlled study. It was shown to accelerate viral clearance (reduction from 102 hours to 90 hours, P = 0.027), but did not reduce fever duration. There were no adverse events observed in this study.[26]

Regarding SARS-CoV-2, an in-vitro study showed that the D11AX22 molecule (ivermectin) reduced viral RNA 5000 times in a course of 48 hours and reduced viral RNA by 99.8% compared to cell cultures treated with DMSO.[27] So far there have been no published randomized clinical trials demonstrating its efficacy in humans with SARS-CoV-2, but an observational case-control study matched by propensity analysis in 1408 subjects (704 who

received a single dose of 150 mcg/kg of ivermectin vs 704 who did not receive it) demonstrated an association between the use of the D11AX22 molecule (ivermectin) and a reduction in in-hospital mortality from 8.5% to 1.4% (Hazard Ratio 0.2, IC95% 0.11-0.37, P<0.0001). Furthermore, in subjects who required mechanical ventilation, there were fewer subjects who died in the group with the D11AX22 molecule (ivermectin = (7.3% vs 21.3%, p <0.001.[28]

Another possible mechanism of action would be by enhancing the immune system as has been described for onchocerciasis and psoriasis in humans.[29-33]

The half-life of the D11AX22 (Ivermectin) molecule in plasma is at least 16 hours. It is absorbed mainly in the intestine and passes into the bloodstream after being administered orally, reaching a therapeutic peak 4 hours after oral administration. It is metabolized in the liver and its metabolites are almost exclusively excreted in the feces, less than 1% of the dose is excreted in the urine.[14] The recommended therapeutic doses as antiparasitic vary between 0.05 and 0.40 mg/kg without undesirable effects or risk to human life; When administered in doses of 0.20 mg/kg, the maximum plasma concentrations reached after four hours are 20 ng/ml, while toxic doses are on the order of 6.6 to 8.6 mg/kg, which can lead to vomiting, blurred vision , mydriasis, ataxia, tremor, and coma. Lethal doses are on the order of 24 mg/kg. No contraindications for its use in humans or negative effects on reproduction or pregnancy have been described, and at least 250 million people in the world have received different doses of this molecule. All of the above allows us to consider the D11AX22 (Ivermectin) molecule as a very safe drug in humans, in whom low toxicity has been seen, unlike other animal species.[34]

Thus, taking into account the in vitro inhibitory capacity against different types of viruses, including SARS CoV2, the encouraging clinical data in humans with COVID 19, the extensive experience in humans that has demonstrated safety using this drug for other pathologies, and the extensive use of the molecule D11AX22 (ivermectin) by this group of investigators,[35-39] there is sufficient rationale to evaluate its use in adults with mild COVID-19 illness.

Given the high morbidity that this disease generates, and the urgent need to find strategies that allow us to reduce disruption in our current lifestyle through safe measures, we propose a double-blind, randomized, placebo-controlled clinical trial to evaluate the efficacy of the D11AX22 (ivermectin) molecule in adults with mild COVID-19 disease.

3. OBJECTIVES

3.1. General objective

To describe the efficacy of the D11AX22 (ivermectin) molecule in adults with mild COVID-19 disease

3.2. Specific objectives

- To compare the time between randomization and clinical deterioration according to a 7-point ordinal scale in subjects receiving the D11AX22 molecule (ivermectin) vs. Placebo.

- To compare the clinical status on specific days of the disease, in subjects receiving the D11AX22 molecule (ivermectin) vs. Placebo.
- To compare the need and duration of hospitalization, supplemental oxygen, ICU, in the two study groups.
- To assess the safety of the D11AX22 (ivermectin) molecule in subjects with mild COVID-19 disease.

4. METHODS

4.1. Description of the Study

4.1.1. Study design

Double-blind, randomized, placebo-controlled clinical trial to evaluate the efficacy of the D11AX22 (ivermectin) molecule in adult subjects with mild SARS CoV2 / COVID 19 disease at participating institutions in the city of Cali, Colombia.

4.1.2. Study Site

The study will be carried out by the Center for Studies in Pediatric Infectology (CEIP) S.A.S. CEIP is a private research center with more than 20 years of experience in conducting individual or multicenter clinical studies with a special interest in epidemiological studies and clinical trials. In total, more than 30 clinical studies have been conducted including 20 multicenter clinical trials and 7 epidemiological monitoring studies. More than 18,000 individuals have been randomized and more than 80,000 participants have received active monitoring.

4.1.3. Study Population

We will invite 400 subjects over 18 years of age with positive molecular test of respiratory samples presenting mild disease by SARS CoV2 / COVID 19. Research participants will be identified through the databases of positive reports for COVID 19 from the laboratories that report to the Valle del Cauca Departmental Health Secretariat.

4.1.4. Intervention

The subjects will be divided into 2 groups, which will be randomized 1: 1

Group 1: Study participants over 18 years of age with mild illness due to SARS CoV2 / COVID 19 who consult at participating institutions in the city of Cali and are randomized to receive ivermectin, once a day for 5 days.

Treatment name: D11AX22 (Ivermectin) Molecule
Drug Name: Ivermectin
Presentation: oral drops in suspension 6 mg/ml.

Route of Administration: Oral
Primary container: 5mL bottle
Volume to be administered: Depends on the subject's weight
Dose: 300 mcg/kg/day
Storage conditions on the label: Room temperature
Indications for administration: It is recommended to take on an empty stomach, preferably a single dose on an empty stomach, with water
Serious interactions: Warfarin, erdafitinib, quinidine

These doses or higher have been used previously and for longer periods of time for other pathologies demonstrating adequate tolerance.[37,40,41]

Group 2: Study participants over 18 years of age with mild illness due to SARS CoV2 / COVID 19 who consult at participating institutions in the city of Cali and are randomized to receive a placebo.

Treatment name: Placebo
Drug Name: 5% dextrose
Presentation: solution
Route of Administration: Oral
Primary container: 5mL bottle
Volume to be administered: Depends on the subject's weight
Dose: same volume that would be administered of Ivermectin to maintain the blind
Storage conditions on the label: Room temperature
Indications for administration: Same as ivermectin.

The active drug and the placebo will be re-labeled to keep study participants and study members blinded. The only person not blinded to the study procedures will be the pharmaceutical chemist who will randomize the study participants.

4.1.5. Outcomes

4.1.5.1. Primary outcome to be evaluated

To determine the time until worsening in 2 or more points in the 7-point ordinal scale in the two study groups.

4.1.5.2. Secondary outcomes to be evaluated

- To determine the clinical state, using the 7-point ordinal scale, on days 2, 5, 8, 11, 15, and 21.
- The proportion of subjects requiring hospitalization, use of supplemental oxygen> 24 hours, admission to the ICU, or dying within 21 days in the two trial groups.
- The duration of supplemental oxygen in each arm of the study.

- The duration of hospitalization in each arm of the study.
- The duration of stay in the ICU in each arm of the study.
- Proportion of subjects who develop adverse events associated with the study drug.
- Proportion of subjects who required discontinuation of medication due to adverse events.

4.1.5.3. Definitions

SARS-CoV-2/COVID-19 Mild illness : Outpatient, or inpatient with supplemental oxygen NOT NEEDING high-flow nasal oxygen, non-invasive mechanical ventilation, or mechanical ventilation.

Severe pneumonia due to SARS-CoV-2/COVID-19 : Inpatient with high-flow nasal cannula oxygen, non-invasive mechanical ventilation or needing mechanical ventilation or ECMO

7-point Clinical Scale:

This scale has been used in clinical studies in subjects with COVID-19 and influenza.[43,44] The seven-point clinical scale has the following categories:

1. Non-hospitalized subject, able to perform daily activities
2. Non-hospitalized subject, unable to perform daily activities
3. Hospitalized, no need for supplemental oxygen
4. Hospitalized, in need of supplemental oxygen
5. Hospitalized, in need of high-flow nasal oxygen or non-invasive mechanical ventilation
6. Hospitalized, in need of mechanical ventilation or ECMO
7. Death

4.1.6. Other variables

In the data collection form, the baseline characteristics of the research participants will be measured, including sociodemographic variables, comorbidities and the “NEWS-2 score” that will be adjusted for secondary analyzes if they are associated with the outcomes.

4.2. Selection of the Study Population

4.2.1 Inclusion criteria:

- Adult subjects over 18 years of age.
- SARS CoV2 / COVID 19 disease confirmed by RT-PCR in any of the laboratories that report to the Departmental Health Secretary, approved for the diagnosis of COVID-19 by the National Institute of Health.
- Onset of SARS CoV2 / COVID 19 illness 5 days ago or less.

- Subjects with mild disease.
- Informed consent signature.

4.2.2 Exclusion criteria:

- Medical history of liver disease.
- History of allergy to ivermectin or any of its components.
- Belonging to another clinical trial that evaluates the efficacy of an investigational drug against COVID-19. The use of other treatments outside of clinical trials is allowed.
- Severe pneumonia at the time of randomization.
- Pregnant or nursing women.
- Subjects receiving Warfarin, erdafitinib, or quinidine.
- Subjects who have received the D11AX22 molecule (ivermectin) within 5 days prior to randomization.
- Inability to evaluate recent liver enzymes
- Elevation in basal liver enzymes> 1.25 times the normal level.

4.2.3 Early withdrawal from the study:

Subjects will be withdrawn from the study:

1. In the event that the investigation product is discontinued within 24 hours of randomization.

4.2.4 Randomization:

Central 1: 1 randomization in blocks of 4 will be generated by a list of random numbers, a separate staff of the telephone allocation system will assign treatment to each of the research participants on a first-come, first-served basis.

4.3. Procedures

4.3.1. Data collection and quality of information

Once the approval of the ethics committee and INVIMA has been obtained, the screening and randomization process will be carried out by a team made up of the study investigators and those they designate. This team will be previously trained in every detail of the protocol. The inclusion of research participants and the collection of their data will be done consecutively as cases are identified in the city of Cali through the database of the laboratory of the Departmental Health Secretariat.
To collect the information, a pre-designed format will be used exclusively for this purpose, that includes the variables necessary to meet the objectives of the study.
This information will be entered into a database by trained personnel with extensive experience in this process.

Data input to the database will be detailed, performing range checks and ensuring consistency and veracity.

4.3.2. Study Procedures

The following procedures have been designed to meet the objectives of the study, reducing interaction between study personnel and research participants with COVID-19:

Once a subject with a positive RT-PCR for SARS-CoV-2 / COVID-19 has been identified, the principal or sub-investigator will contact the subject by telephone to provide study information through the telephone informed consent process. Subjects who express interest will be surveyed to obtain clinical and socio-demographic data and will be personally visited at their home or hospitalization site to sign the informed consent form in the presence of two witnesses.

At this initial visit, demographic data will be confirmed, informed consent will be signed and a blood sample will be obtained for liver enzyme evaluation.

The next day, it will be determined whether or not the subject meets the selection criteria, including verification of liver enzyme result and pregnancy test (for women of childbearing age). Once it is defined that the subject meets the selection criteria, the participant will be randomized, the necessary clinical data will be obtained, vital signs will be taken, and the investigation product will be initiated.

The administration of the research product will be done by self-administration in case of outpatient research participants, and in the case of hospitalized participants the administration instructions will be given to the subject and / or the health personnel who attends him/her, complying with the requirements of the institution.

Research participants will be contacted daily by phone call to remind them to take the research product, assess drug adherence, and detect adverse events to the research product.

4.3.3 Table of visits and follow-ups

Visit number (V)	Visit1	Visit 2	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]
Study deadlines	**Day (D) 0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D8**	**D11**	**D 15**	**D 21**
Intervals (days)			**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**
Signed informed consent form	**X**									
Inclusion/exclusion criteria		**X**								
Assignment of the research participant number		**X**								
Demographic Data	**X**									
Medical background check		**X**								
Vital signs and pulse oximetry		**X**								
Calculation of NEWS -2 score:		**X**								
Collection of clinical data		**X**								
Urine sample to evaluate pregnancy		**X**								
Blood Sample to evaluate Hepatic enzymes	**X**									
Randomization		**X**								
Dispensing and initiating medication/placebo		**X**								
Evaluation of the 7-point ordinal scale		**X**	**X**			**X**	**X**	**X**	**X**	**X**
Assessment of secondary outcomes - Pt. at home or hospitalized? - Has required oxygen? - Has required ICU? - Vital status		**X**	**X**			**X**	**X**	**X**	**X**	**X**

Collection of Serious Adverse Events (SAE)			x			x	x	x	x	x
Pharmacotherapeutic Adherence		x	x	x	x	x				
Remember the need to take the investigation product			x	x	x	x				
Collect symptoms reported to the admin of the investigation product			x	x	x	x	x	x	x	x
Record of completion										x

[1] In the case of hospitalized research participants, the study staff will personally visit the subject and/or contact the physician in charge by telephone, and/or through the medical history will obtain the necessary information and thus be able to determine primary or secondary outcomes

4.3.4 Masking and blinding

Placebo will be administered to half of the research participants. The placebo will consist of 5% dextrose, which will be labeled to give it the same appearance as the investigational product.

4.3.5 Follow up

To evaluate the evolution of the research participants and determine safety and efficacy according to the primary and secondary outcomes, subjects will be called on days 2, 3, 4, 5, 8, 11, 15 and 21 to fill out a questionnaire. In the event that the research participant requires hospitalization, the research participant will be visited personally to extract the information from the medical record. On the 21st day, the study will finish the evaluation of primary and secondary outcomes. In the case of hospitalized research participants, follow-up will continue until the end of hospitalization to determine their vital status.

4.3.6 Adherence to treatment

The administration of the research product will be done by self-administration in case of outpatient research participants, and in the case of hospitalized participants the administration instructions will be given to the subject and / or the health personnel who attends him/her, complying with the requirements of the institution.

Research participants will be contacted daily by phone call to remind them to take the research product, assess drug adherence, and detect adverse events to the research product.

4.3.7 Measurement of Security Parameters

On the day of visit 01 (day 0), all participants will have their liver enzymes measured. The research participant will not be randomized and therefore will not be included in the study if these are elevated> 1.25 times normal values.
In follow-up calls (days 2, 3, 4, 5, 8, 11, 15, and 21) ,research participants will be asked about symptoms that have historically been reported in subjects receiving ivermectin (uncontrollable shaking of a body part, dizziness, loss of appetite, nausea, vomiting, stomach pain or bloating, diarrhea, constipation, weakness, drowsiness, chest discomfort, swelling (face, arms, hands, feet, ankles, or lower legs), and other symptoms, pain and swelling in joints, painful and swollen glands in the neck, armpit, or groin, fast heartbeat, eye pain, blurred vision, redness or tearing, swelling (face, arms, hands, feet, ankles or calves), pain and swelling in the joints, painful and swollen glands in the neck, armpit or groin, fast heartbeat, pain in the eyes, blurred vision, redness or tearing, swelling of the eyes or eyelids, abnormal sensation in the eyes, fever, blisters or peeling of skin, rash, hives, and itching) to complete the information requested by the data collection form.
The proportion of research participants with adverse events in both arms will be compared.

4.4 Sample size

According to the literature, 20% of patients will develop the primary outcome (worsening of 2 or more points in the 7-point ordinal scale). Thus, we will need to include 400 patients (72 total events plus 10% lost events) in order to detect a Hazard ratio of 0.5 of ivermectin vs. placebo in time to deterioration, with a power of 80% and alpha of 0.05

4.5 Data analysis

An interim analysis will be conducted without breaking the blind when 200 research participants have been randomized. The study will be discontinued if this analysis detects a significant treatment effect on the main objective, if it is detected that a significantly larger group of subjects withdraw from the intervention arm due to adverse events, or if no difference is detected between the two groups. Recruitment will continue while this analysis is performed.

The information will be analyzed with Stata version 14 (SE; Stata corporation, College Station, Texas). For this purpose, subjects will be grouped into 2 groups that comprise

Group 1: Subjects who received ivermectin
Group 2: Subjects who received placebo.

The baseline characteristics of the subjects will be described by descriptive analysis.

The main analysis will be by intent-to-treat. The time until worsening of two or more points will be determined when all research subjects reach day 21. Clinical deterioration or death before the 21st will be right sensored- on the 21st. Deaths will be included unless there is strong evidence that the cause of death was not associated with COVID-19. The time until worsening of two or more points will be presented in a Kaplan-Meier curve and will be compared with the log-rank test. Hazard Ratios will be calculated with their 95% confidence interval through a Cox proportional-hazards model.

Secondary outcomes and adverse events in each of the groups will be expressed by absolute and relative frequencies with their corresponding 95% confidence interval. For the univariate analysis in the quantitative variables, statistical parameters of central tendency and dispersion (means and standard deviations) will be described if the variable has a normal distribution. In case of not having a normal distribution, medians and interquartile ranges will be described. The qualitative variables will be measured and analyzed through proportions with their corresponding 95% confidence interval.

To determine the efficacy of the intervention in secondary outcomes, a bivariate analysis will be performed, evaluating the association of treatment, demographic and clinical variables with the different outcomes.

5. ETHICAL CONSIDERATIONS

5.1. Ethical conduct of the study

The conduct of this study will be in accordance with all local and/or national regulations and directives.

In accordance with international guidelines, this study is considered to comply with the following principles to protect research subjects:

1. Social and clinical value: SARS CoV2 is so far a virus with high rates of morbidity and mortality, but without specific treatment. Demonstrating the effectiveness of a safe treatment in the early stages could mitigate the negative impact that this pathology currently has.

2. Scientific validity: There is no therapy so far that has been proven to be effective for this pathology in its initial stages. Ivermectin has biological plausibility and an observational study in patients with SARS-CoV-2 suggests clinical utility.

3. Fair selection of research subjects: Individuals with a confirmed diagnosis of SARS CoV 2 are chosen. Randomization will guarantee a homogeneous distribution of confounders in both arms.

4. Risk-benefit ratio - favorable: Ivermectin is a safe drug, widely used in humans with different pathologies, so we do not anticipate adverse events. The potential benefit to subjects receiving the drug may be greater in mitigating SARS-CoV-2 disease. All subjects will be contributing to the

development of science at this time of global emergency, by providing information aimed at improving the management of this disease.

5. Informed consent: All subjects must accept their participation in the study by signing an informed consent form and can withdraw their participation at any time.

6. Participation in the study does not generate any economic benefit for the subject, no incentives will be given and since there is no mobilization, transportation and food expenses are not covered.

5.2. Source data and documents

The "source data" is the data contained in the source documents. Source documents are original documents or certified copies and include, but are not limited to, memory aids, medical and hospital records, pre-selection records, informed consent/assent forms, telephone contact records, and worksheets. The purpose of the study source documents is to document the existence of the subjects, and to support the integrity of the data collected in the study. Investigators should maintain the source documents of the study so that they are accurate and legible, and are complete and up-to-date.

The investigator and center staff handling source documents must follow good documentation practices.

5.3. Confidentiality of data and access to participant records

In the event that a subject's medical history is not at the research center, it is the responsibility of the investigator to obtain said history if necessary.

All personal data collected related to subjects, investigators or anyone involved in the study, which may be included in the sponsor's database, will be treated in accordance with all applicable laws and regulations. The data collected should be adequate, relevant and not excessive in relation to the purposes for which it is collected. Each category of data must be adequately justified and be in line with the objective of the study.

Subjects will be assigned a unique identifier by the investigator. Any subject record or data set that is transferred will contain only the identifier; the names of the subject or any information that could make the subject identifiable will not be transferred.

The subjects must be informed that their personal data related to the study will be used by the investigator, in accordance with local data protection laws. The level of disclosure should also be explained to the subject.

The subject must be informed that their medical history may be examined by Quality Management auditors or other authorized persons designated by the investigator, by members of CEI, and by inspectors from regulatory authorities.

When filing or processing personal data relevant to the investigator and/or subjects, all appropriate measures will be taken to safeguard and prevent access to this data by an unauthorized third party.

5.4. Audits and inspections

The Quality Management area or an independent auditor can carry out a quality assurance audit at any time, to verify that the study has been carried out following the protocol and other applicable regulations. Regulatory authorities may conduct an inspection. The investigator must allow direct access to study documents during these inspections and audits.

5.5. Archive

The investigator must keep all study documents after its completion or interruption, for as long as the applicable laws and regulations require. The study documents will be kept for at least 25 years. Study personnel must not destroy or allow the destruction of any study document without written notice.

Archived data may be kept in electronic records, as long as a backup copy is available, and a hard copy can be obtained upon request. The protocol, documentation, approvals, and all other documents related to the study will be kept by the investigator in the CEIP Archive. All documents and data will be made available to the relevant authorities upon request.

5.6. Financial contract and insurance coverage

All parties involved in the conduct of the study will sign a study agreement, if applicable. CEIP has an insurance policy that covers any liability that may arise from the use of the study protocol.

5.7. Publishing policy

The data obtained from this study are the exclusive property of CEIP. Any publication or presentation related to the study should be sent to CEIP for review before the manuscript is submitted. After publication of study results, any participating center may publish or otherwise use its own data, as long as any publication of study data acknowledges the study group. In addition, CEIP will be offered association with all publications of this nature, with the understanding that CEIP has the right to reject such association.

6. EXPECTED RESULTS

After this study, we hope to understand the efficacy and safety of ivermectin for the treatment of adult patients with mild SARS CoV 2 disease.

7. BIBLIOGRAPHY

1. Corman VM, Lienau J, Witzenrath M. [Coronaviruses as the cause of respiratory infections]. *Internist (Berl).* 2019;60(11):1136-1145.
2. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol.* 2016;14(8):523-534.
3. Fehr AR, Channappanavar R, Perlman S. Middle East Respiratory Syndrome: Emergence of a Pathogenic Human Coronavirus. *Annu Rev Med.* 2017;68:387-399.
4. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497-506.
5. 2020; https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6. Accessed April 25, 2020.
6. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet.* 2020;395(10224):565-574.
7. Zhang J, Litvinova M, Wang W, et al. Evolving epidemiology and transmission dynamics of coronavirus disease 2019 outside Hubei province, China: a descriptive and modelling study. *Lancet Infect Dis.* 2020.
8. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal.* 2020.
9. Velavan TP, Meyer CG. The COVID-19 epidemic. *Trop Med Int Health.* 2020;25(3):278-280.
10. Departamental SdS. Comunicación Personal. 2020.
11. C.A S. Consenso colombiano de atencion, diagnóstico y manejo de la infección por SARS-CoV-2/COVID-19 en establecimientos de atención de la salud. *INFECTIO.* 2020;24.
12. Uyeki TM, Bernstein HH, Bradley JS, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenzaa. *Clin Infect Dis.* 2019;68(6):895-902.
13. King CL. Is ivermectin safe in pregnancy? *Lancet Glob Health.* 2020;8(1):e12-e13.
14. Campbell WC. *Ivermectin and Abamectin.* 1989.
15. Omura S. Ivermectin: 25 years and still going strong. *Int J Antimicrob Agents.* 2008;31(2):91-98.
16. Crump A, Omura S. Ivermectin, 'wonder drug' from Japan: the human use perspective. *Proc Jpn Acad Ser B Phys Biol Sci.* 2011;87(2):13-28.
17. Yang SN, Atkinson SC, C. W, al. e. The broad spectrum antiviral ivermectin targets the host nuclear transport importin α/β heterodime. *Antiviral research.* 2020.
18. Wagstaff KM, Sivakumaran H, Heaton SM, Harrich D, Jans DA. Ivermectin is a specific inhibitor of importin alpha/beta-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochem J.* 2012;443(3):851-856.

19. Mastrangelo E, Pezzullo M, De Burghgraeve T, et al. Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. *J Antimicrob Chemother.* 2012;67(8):1884-1894.
20. Tay MY, Fraser JE, Chan WK, et al. Nuclear localization of dengue virus (DENV) 1-4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin. *Antiviral Res.* 2013;99(3):301-306.
21. Lundberg L, Pinkham C, Baer A, et al. Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan Equine Encephalitis Virus replication. *Antiviral Res.* 2013;100(3):662-672.
22. Atkinson SC, Audsley MD, Lieu KG, et al. Recognition by host nuclear transport proteins drives disorder-to-order transition in Hendra virus V. *Sci Rep.* 2018;8(1):358.
23. Shechter S, Thomas DR, Lundberg L, et al. Novel inhibitors targeting Venezuelan equine encephalitis virus capsid protein identified using In Silico Structure-Based-Drug-Design. *Sci Rep.* 2017;7(1):17705.
24. Caly L, Wagstaff KM, Jans DA. Nuclear trafficking of proteins from RNA viruses: potential target for antivirals? *Antiviral Res.* 2012;95(3):202-206.
25. Jans DA, Martin AJ, Wagstaff KM. Inhibitors of nuclear transport. *Curr Opin Cell Biol.* 2019;58:50-60.
26. E Y, P A, D M. Efficacy and Safety of Ivermectin against Dengue Infection: A Phase III, Randomized, Double blind, Placebo-controlled Trial. Paper presented at: The 34th Annual Meeting The Royal College of Physicians of Thailand; 26th - 28th April 2018,, 2018; PEACH Royal Cliff Beach Resort, Pattaya, Chonburi, Thailand.
27. L C, J D, M C, al. e. The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Research.* 2020.
28. A. P, S. D, D. G, MR M. Usefulness of Ivermectin in COVID-19 Illness. 2020.
29. Steel C, Lujan-Trangay A, Gonzalez-Peralta C, Zea-Flores G, Nutman TB. Immunologic responses to repeated ivermectin treatment in patients with onchocerciasis. *J Infect Dis.* 1991;164(3):581-587.
30. Knab J, Darge K, Buttner DW. Immunohistological studies on macrophages in lymph nodes of onchocerciasis patients after treatment with ivermectin. *Trop Med Int Health.* 1997;2(12):1156-1169.
31. Soboslay PT, Dreweck CM, Hoffmann WH, et al. Ivermectin-facilitated immunity in onchocerciasis. Reversal of lymphocytopenia, cellular anergy and deficient cytokine production after single treatment. *Clin Exp Immunol.* 1992;89(3):407-413.
32. Akuffo H, Maasho K, Lavebratt C, Engstrom K, Britton S. Ivermectin-induced immunopotentiation in onchocerciasis: recognition of selected antigens following a single dose of ivermectin. *Clin Exp Immunol.* 1996;103(2):244-252.
33. Semnani RT, Keiser PB, Coulibaly YI, et al. Filaria-induced monocyte dysfunction and its reversal following treatment. *Infect Immun.* 2006;74(8):4409-4417.
34. Dobson RJ, Besier RB, Barnes EH, et al. Principles for the use of macrocyclic lactones to minimise selection for resistance. *Aust Vet J.* 2001;79(11):756-761.
35. Costa JL, Diazgranados JA. Ivermectin for spasticity in spinal-cord injury. *Lancet.* 1994;343(8899):739.
36. Diazgranados JA, Costa JL. Deaths after ivermectin treatment. *Lancet.* 1997;349(9066):1698.

37. J D, J M-F, L C-G. Ivermectina como coadyuvante en la epilepsia refractaria. *Rev Neurol.* 2017;65:303-310.
38. Diazgranados-Sanchez JA. [Potential interactions with ivermectin as adjuvant therapy for refractory epilepsy. Reply]. *Rev Neurol.* 2018;66(7):251-252.
39. Diazgranados-Sanchez JA, Barrios-Arrazola G, Costa JL, Burbano-Pabon J, Pinzon-Bedoya J. [Ivermectin as a therapeutic alternative in neurocysticercosis that is resistant to conventional pharmacological treatment]. *Rev Neurol.* 2008;46(11):671-674.
40. Smit MR, Ochomo EO, Aljayyoussi G, et al. Safety and mosquitocidal efficacy of high-dose ivermectin when co-administered with dihydroartemisinin-piperaquine in Kenyan adults with uncomplicated malaria (IVERMAL): a randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis.* 2018;18(6):615-626.
41. Juarez M, Schcolnik-Cabrera A, Duenas-Gonzalez A. The multitargeted drug ivermectin: from an antiparasitic agent to a repositioned cancer drug. *Am J Cancer Res.* 2018;8(2):317-331.
42. Guzzo CA, Furtek CI, Porras AG, et al. Safety, tolerability, and pharmacokinetics of escalating high doses of ivermectin in healthy adult subjects. *J Clin Pharmacol.* 2002;42(10):1122-1133.
43. Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. *New England Journal of Medicine.* 2020.
44. Fan HW, Han Y, Liu W, et al. [A randomized controlled study of peramivir, oseltamivir and placebo in patients with mild influenza]. *Zhonghua Nei Ke Za Zhi.* 2019;58(8):560-565.

DOUBLE BLIND, RANDOMIZED, CONTROLLED CLINICAL TRIAL TO INVESTIGATE THE EFFECTIVENESS OF THE D11AX22 MOLECULE IN ADULT SUBJECTS FROM VALLE DEL CAUCA WITH INITIAL STAGES OF INFECTION BY SARS COV2 / COVID-19

Protocol Amendment Date: Final Amendment 4, October 27, 2020

Protocol Amendment Date: Final Amendment 3, September 16, 2020

Protocol Amendment Date: Final Amendment 2, August 04, 2020

Protocol Amendment Date: Final Amendment 1: June 9, 2020

Protocol Date: Final Version 2.0: May 12, 2020

PRINCIPAL INVESTIGATOR

Eduardo López Medina, MD MSc
Center for Studies in Pediatric Infectology
Universidad del Valle
Imbanaco Medical Center

DIRECTION AND CONDUCT OF THE TRIAL

Centro de Estudios en Infectología Pediátrica --CEIP-- S.A.S

TRIAL SPONSOR

Centro de Estudios en Infectología Pediátrica --CEIP-- S.A.S
Calle 5 B 5 No. 37Bis - 20
Cali Valle
COLOMBIA

Investigator Agreement to Protocol

I agree with:

- Conducting the study in compliance with this protocol, any future modifications to the protocol or mutually agreed administrative changes to the protocol, the terms of the study agreement, and any other study development procedures and/or study development documents.
- Assuming responsibility for the proper conduct of the study on this site.
- Being aware of and comply with the "Good Clinical Practices" (GCP) and all applicable regulatory requirements.
- Ensuring that all persons assisting me with the study are adequately informed regarding the duties and functions related to the study as described in the protocol.
- Acquiring reference ranges for laboratory analyzes performed locally and, if required by local regulations, obtain current laboratory certification or the Quality Assurance Procedure Manual.
- Ensuring that no samples (including serum samples) are retained at the site or elsewhere without the express written informed consent of the research participant.
- Not carrying out other biological tests on the samples, except those described in the protocol or its modification (s).

1. INTRODUCTION

Since the discovery of human coronaviruses (HCoV) in the 1960s, 6 viruses, including HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV (severe acute respiratory syndrome) and MERS-CoV (Middle East respiratory syndrome), have been recognized as agents causing respiratory infections, some causing severe life-threatening multisystemic syndromes. HCoV NL63 and HCoV HKU1 were described in 2004 and 2005, respectively, and together with HCoV 229E and OC43 are responsible for up to 35% of upper respiratory infections, generally in epidemic outbreaks.[1]

HCoV OC43 is the most prevalent, affecting mostly children under 5 years of age. It is very frequently identified in coinfections with other respiratory viruses, which makes it difficult to determine its true role. In addition, these have been associated with more serious conditions, which lead to hospitalization and even fatal cases. Several members of the Coronaviridae family are constantly circulating in the human population and generally cause mild respiratory illness.[1]

The most severe disease-causing coronaviruses in humans are SARS-CoV and MERS-CoV, both of which are transmitted from animals to humans and cause severe respiratory illness.[2,3]

At the end of 2019, a series of cases of severe acute respiratory infections with characteristics that had not been previously described were identified in China. Later it was shown that these characteristics were produced by a new coronavirus that was called SARS-CoV-2, which causes COVID-19.[4] The virus spread rapidly throughout the world, causing more than 2,200,000 cases by April 25, 2020, with nearly 100,000 deaths.[5]

It is a single-stranded RNA virus, which makes it easy to mutate, rapidly adapting to new hosts. It belongs to the Beta coronavirus family and can infect type 2 pneumocytes and ciliated bronchial epithelial cells producing an acute respiratory disease whose most common symptoms are fever, cough, nausea or vomiting and diarrhea, with varying degrees of severity, producing severe illness in older subjects and those with associated comorbidities such as high blood pressure, heart problems or diabetes.[6] About 80% of people recover from the disease without the need for any specific treatment, however, 1 in 5 people who contract the virus progress to severe illness.[7]

The diagnosis is made by identifying the genetic material of the virus by real-time polymerase chain reaction (RT-PCR) of the affected airways. Among the limitations of this test is that the SARS-CoV2 infection can only be determined at the time of the test. We cannot know if the subject was infected days before it. Furthermore, although RT-PCR is a relatively reliable technique, the sensitivity depends on the way the sample to be processed is obtained.[8] In August 2020, the Colombian National Institute of Health included in the diagnostic guidelines for COVID-19 the use of SARS-CoV-2 antigen detection in nasopharyngeal samples. This test has proven to be highly specific and allows to have results in minutes, which facilitates the rapid diagnosis of the disease. [45]

Because it is a new virus, we do not have previous immunity to it, which makes us susceptible to infection. We also do not have antiviral drugs or effective vaccines that can mitigate the infection.[9] Currently, there are hundreds of clinical studies looking for the best therapeutic strategies, but no randomized clinical study has yet demonstrated effective

therapeutic strategies in the early stages of infection. Given the high morbidity and mortality that this disease generates in its most advanced stages, the global urgency to find measures that reduce the impact of this disease and the delay that represents the generation of new antiviral drugs, it is essential to evaluate in a clinical trial whether already available drugs that have demonstrated safety and benefit in observational or in-vitro studies against SARS-CoV-2, maintain their usefulness in early stages of COVID-19.

2. PROBLEM STATEMENT AND JUSTIFICATION

Valle del Cauca is the department with the second highest number of cases and lethality in Colombia. As of April 26, 2020, we have 841 cases, of which 12% have required hospitalization and 8.7% require intensive care, with a fatality rate of 6.5%.[10] Every day a greater number of cases are reported in Valle del Cauca.

In the early or mild phases of the infection, the currently recommended procedure is symptom control, with no medication proven to reduce disease progression.[11] However, about 20% of subjects progress to severe disease, so it is urgent to identify interventions that mitigate this progression. This is especially important considering that, as with other antiviral medications, early reduction in viral load during mild forms of the disease has favorable effects on its outcome.[12]

The D11AX22 molecule (ivermectin) is a new therapeutic proposal against COVID-19. The D11AX22 molecule (ivermectin) is one of the most important drugs ever discovered and has been recognized as a benchmark by the American Chemical Society. Its discoverers were awarded the Nobel Prize in 2015 for demonstrating its great efficacy as a broad-spectrum antiparasitic.[13] Originally introduced as an antiparasitic in veterinary medicine in the early 1980s, it is a drug that eliminates a wide range of endo and ectoparasites in livestock and other animals. For more than 35 years it has been used in humans with great efficacy and safety to treat endoparasites that are difficult to control, such as filariasis, onchocerciasis or strongyloidiasis, also ectoparasites, such as pediculosis capitis and myiasis.[14-16]

Its antiviral effect has recently been evaluated against SARS-CoV-2 and other viruses. The IMP heterodimer$\alpha\beta$ 1 is a molecule that binds to the viral load protein and transports it to the nucleus, reducing the antiviral capacity of the host cell. The D11AX22 (Ivermectin) molecule destabilizes this heterodimer, prevents its binding to the viral protein, and prevents its entry into the host cell nucleus.[17] Other actions of the D11AX22 (ivermectin) molecule include the ability to inhibit nuclear importation of integrase and HIV viral replication,[18] inhibition of dengue virus NS3 helicase (DENV)[19]; and it has been shown to be a potent inhibitor of the yellow fever virus replication through its action on the viral helicase, preventing the synthesis of viral RNA.[19] The D11AX22 (ivermectin) molecule has also been shown to inhibit the importation of different proteins into the nucleus, including tumor antigen (T-ag) from simian virus SV40 and DENV NS5, and limits infection by other viruses, including DENV serotypes 1-4 and influenza[18,20 ,21 ,22,23] due to the dependence of RNA virus on IMP$\alpha\beta$ 1 during infection.[24,25] The D11AX22 molecule (Ivermectin) was evaluated as a treatment in humans with dengue in a phase 3, randomized, double-blind, placebo-controlled study. It was shown to accelerate viral clearance (reduction from 102 hours to 90 hours, P = 0.027), but did not reduce fever duration. There were no adverse events observed in this study.[26]

Regarding SARS-CoV-2, an in-vitro study showed that the D11AX22 molecule (ivermectin) reduced viral RNA 5000 times in a course of 48 hours and reduced viral RNA by 99.8%

compared to cell cultures treated with DMSO.[27] So far there have been no published randomized clinical trials demonstrating its efficacy in humans with SARS-CoV-2, but an observational case-control study matched by propensity analysis in 1408 subjects (704 who received a single dose of 150 mcg/kg of ivermectin vs 704 who did not receive it) demonstrated an association between the use of the D11AX22 molecule (ivermectin) and a reduction in in-hospital mortality from 8.5% to 1.4% (Hazard Ratio 0.2, IC95% 0.11-0.37, P<0.0001). Furthermore, in subjects who required mechanical ventilation, there were fewer subjects who died in the group with the D11AX22 molecule (ivermectin = (7.3% vs 21.3%, p <0.001.[28]

Another possible mechanism of action would be by enhancing the immune system as has been described for onchocerciasis and psoriasis in humans.[29-33]

The half-life of the D11AX22 (Ivermectin) molecule in plasma is at least 16 hours. It is absorbed mainly in the intestine and passes into the bloodstream after being administered orally, reaching a therapeutic peak 4 hours after oral administration. It is metabolized in the liver and its metabolites are almost exclusively excreted in the feces, less than 1% of the dose is excreted in the urine.[14] The recommended therapeutic doses as antiparasitic vary between 0.05 and 0.40 mg/kg without undesirable effects or risk to human life; When administered in doses of 0.20 mg/kg, the maximum plasma concentrations reached after four hours are 20 ng/ml, while toxic doses are on the order of 6.6 to 8.6 mg/kg, which can lead to vomiting, blurred vision , mydriasis, ataxia, tremor, and coma. Lethal doses are on the order of 24 mg/kg. No contraindications for its use in humans or negative effects on reproduction or pregnancy have been described, and at least 250 million people in the world have received different doses of this molecule. All of the above allows us to consider the D11AX22 (Ivermectin) molecule as a very safe drug in humans, in whom low toxicity has been seen, unlike other animal species.[34]

Thus, taking into account the in vitro inhibitory capacity against different types of viruses, including SARS CoV2, the encouraging clinical data in humans with COVID 19, the extensive experience in humans that has demonstrated safety using this drug for other pathologies, and the extensive use of the molecule D11AX22 (ivermectin) by this group of investigators,[35-39] There is sufficient rationale to evaluate its use in adults with mild COVID-19 illness.

Given the high morbidity that this disease generates, and the urgent need to find strategies that allow us to reduce disruption in our current lifestyle through safe measures, we propose a double-blind, randomized, placebo-controlled clinical trial to evaluate the efficacy of the D11AX22 (ivermectin) molecule in adults with mild COVID-19 disease.

3. OBJECTIVES

3.1. General objective

To describe the efficacy of the D11AX22 (ivermectin) molecule in adults with mild COVID-19 disease

3.2. Specific objectives

- To compare the time between randomization and disappearance of symptoms in subjects receiving the D11AX22 molecule (ivermectin) vs. Placebo.
- To compare the time between randomization and clinical deterioration according to a 7-point ordinal scale in subjects receiving the D11AX22 molecule (ivermectin) vs. Placebo.
- To compare the clinical status on specific days of the disease, in subjects receiving the D11AX22 molecule (ivermectin) vs. Placebo.
- To compare the need and duration of hospitalization, supplemental oxygen, ICU, and duration of fever in the two study groups.
- To assess the safety of the D11AX22 (ivermectin) molecule in subjects with mild COVID-19 disease.

4. METHODS

4.1. Description of the Study

4.1.1. Study design

Double-blind, randomized, placebo-controlled clinical trial to evaluate the efficacy of the D11AX22 (ivermectin) molecule in adult subjects with mild SARS CoV2 / COVID 19 disease at participating institutions in Valle del Cauca.

4.1.2. Study Site

The study will be carried out by the Center for Studies in Pediatric Infectology (CEIP) S.A.S. CEIP is a private research center with more than 20 years of experience in conducting individual or multicenter clinical studies with a special interest in epidemiological studies and clinical trials. In total, more than 30 clinical studies have been conducted including 20 multicenter clinical trials and 7 epidemiological monitoring studies. More than 18,000 individuals have been randomized and more than 80,000 participants have received active monitoring.

4.1.3. Study Population

We will invite 476 subjects over 18 years of age with positive molecular test of respiratory samples presenting mild disease by SARS CoV2 / COVID 19. Research participants will be identified through the databases of positive reports for COVID 19 from the laboratories that report to the Valle del Cauca Departmental Health Secretariat.

4.1.4. Intervention

The subjects will be divided into 2 groups, which will be randomized 1: 1

Group 1: Study participants over 18 years of age with mild illness due to SARS CoV2 / COVID 19 who consult at participating institutions in Valle del Cauca and are randomized to receive ivermectin, once a day for 5 days.

Treatment name: D11AX22 (Ivermectin) Molecule
Drug Name: Ivermectin
Presentation: oral drops in suspension 6 mg/ml.
Route of Administration: Oral
Primary container: 5mL bottle
Volume to be administered: Depends on the subject's weight
Dose: 300 mcg/kg/day
Storage conditions on the label: Room temperature
Indications for administration: It is recommended to take on an empty stomach, preferably a single dose on an empty stomach, with water
Serious interactions: Warfarin, erdafitinib, quinidine

These doses or higher have been used previously and for longer periods of time for other pathologies demonstrating adequate tolerance.[37,40,41]

Group 2: Study participants over 18 years of age with mild illness due to SARS CoV2 / COVID 19 who consult at participating institutions in Valle del Cauca and are randomized to receive a placebo.

Treatment name: Placebo
Drug Name: Placebo
Presentation: solution
Route of Administration: Oral
Primary container: 5mL bottle
Volume to be administered: Depends on the subject's weight
Dose: same volume that would be administered of Ivermectin to maintain the blind
Storage conditions on the label: Room temperature
Indications for administration: Same as ivermectin.

The active drug and the placebo will be re-labeled to keep study participants and study members blinded. The only person not blinded to the study procedures will be the pharmaceutical chemist who will randomize the study participants.

4.1.5. Outcomes

4.1.5.1. Primary outcome to be evaluated

To determine the time until the resolution of the symptoms in the two study groups.

4.1.5.2. Secondary outcomes to be evaluated

- To determine the time to deterioration in two or more points of the 7-point ordinal scale in the two study groups.
- To determine the clinical state, using the 7-point ordinal scale, on days 2, 5, 8, 11, 15, and 21.
- The proportion of subjects requiring hospitalization, use of supplemental oxygen> 24 hours, admission to the ICU, or dying within 21 days in the two trial groups.
- The duration of supplemental oxygen in each arm of the study.
- The duration of hospitalization in each arm of the study.
- The duration of stay in the ICU in each arm of the study.
- The duration of fever (axillary temperature> 38C or subjective feeling of fever) in each arm of the study.
- Proportion of subjects who develop adverse events associated with the study drug.
- Proportion of subjects who required discontinuation of medication due to adverse events.

4.1.5.3. Definitions

SARS-CoV-2/COVID-19 Mild illness : Outpatient, or inpatient with supplemental oxygen NOT NEEDING high-flow nasal oxygen, non-invasive mechanical ventilation, or mechanical ventilation.

Severe pneumonia due to SARS-CoV-2/COVID-19 : Inpatient with high-flow nasal cannula oxygen, non-invasive mechanical ventilation or needing mechanical ventilation or ECMO

7-point Clinical Scale:

This scale has been used in clinical studies in subjects with COVID-19 and influenza.[43,44] The seven-point clinical scale has the following categories:

1. Non-hospitalized subject, able to perform daily activities
2. Non-hospitalized subject, unable to perform daily activities
3. Hospitalized, no need for supplemental oxygen
4. Hospitalized, in need of supplemental oxygen
5. Hospitalized, in need of high-flow nasal oxygen or non-invasive mechanical ventilation
6. Hospitalized, in need of mechanical ventilation or ECMO
7. Death

4.1.6. Other variables

In the data collection form, the baseline characteristics of the research participants will be measured, including sociodemographic variables, comorbidities and the “NEWS-2 score” that will be adjusted for secondary analyzes if they are associated with the outcomes.

4.2. Selection of the Study Population

4.2.1 Inclusion criteria:

- Adult subjects over 18 years of age.
- SARS CoV2 / COVID 19 disease confirmed by antigen detection tests authorized by INVIMA or by RT-PCR in any of the laboratories that report to the Departmental Health Secretary, approved for the diagnosis of COVID-19 by the National Institute of Health.
- Onset of SARS CoV2 / COVID 19 illness 7 days ago or less.
- Subjects with mild disease.
- Informed consent signature.

4.2.2 Exclusion criteria:

- Medical history of liver disease.
- History of allergy to ivermectin or any of its components.
- Belonging to another clinical trial that evaluates the efficacy of an investigational drug against COVID-19. The use of other treatments outside of clinical trials is allowed.
- Severe pneumonia at the time of randomization.
- Pregnant or nursing women.
- Subjects receiving Warfarin, erdafitinib, or quinidine.
- Subjects who have received the D11AX22 molecule (ivermectin) within 5 days prior to randomization.
- Inability to evaluate recent liver enzymes, no more than 3 days prior to selection.
- Elevation in basal liver enzymes> 1.5 times the normal level.
- Subject whose contact with study personnel occurs between days 5-7 and at that time manifests significant and progressive improvement ≥ 48 hours in signs and symptoms of COVID-19.

4.2.3 Early withdrawal from the study:

Subjects will be withdrawn from the study:

1. In the event that the investigation product is discontinued within 24 hours of randomization.

4.2.4 Randomization:

Central 1: 1 randomization in blocks of 4 will be generated by a list of random numbers, a separate staff of the telephone allocation system will assign treatment to each of the research participants on a first-come, first-served basis.

4.3. Procedures

4.3.1. Data collection and quality of information

Once the approval of the ethics committee and INVIMA has been obtained, the screening and randomization process will be carried out by a team made up of the study investigators and those they designate. This team will be previously trained in every detail of the protocol. The inclusion of research participants and the collection of their data will be done consecutively as cases are identified in Valle del Cauca through the database of the laboratory of the Departmental Health Secretariat.
To collect the information, a pre-designed format will be used exclusively for this purpose, that includes the variables necessary to meet the objectives of the study.
This information will be entered into a database by trained personnel with extensive experience in this process.
Data input to the database will be detailed, performing range checks and ensuring consistency and veracity.

4.3.2. Study Procedures

The following procedures have been designed to meet the objectives of the study, reducing interaction between study personnel and research participants with COVID-19:

Once a subject with a positive antigen detection test or PCR for SARS-CoV-2 / COVID-19 has been identified, the principal or sub-investigator will contact the subject by telephone to provide study information through the telephone informed consent process. Subjects who express interest will be surveyed to obtain clinical and socio-demographic data and will be personally visited at their home or hospitalization site to sign the informed consent form in the presence of two witnesses.

At the screening visit, demographic data will be confirmed, informed consent will be signed, a blood sample will be obtained for liver enzyme evaluation, a pregnancy test (for women of childbearing age) and the participant's weight will be measured.

At the randomization visit, it will be determined whether or not the subject meets the selection criteria, including verification of liver enzyme result and pregnancy test (for women of childbearing age). Once it is defined that the subject meets the selection criteria. The research participant will be randomized, the necessary clinical data will be obtained, vital signs will be taken, and the investigation product will be initiated.

The administration of the research product will be done by self-administration in case of outpatient research participants, and in the case of hospitalized participants the administration instructions will be given to the subject and / or the health personnel who attends him/her, complying with the requirements of the institution.

Research participants will be contacted daily by phone call to remind them to take the research product, assess drug adherence, and detect adverse events to the research product.

4.3.3 Table of visits and follow-ups

Visit number (V)	Screening	Randomization	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]	Phone call [1]
Study deadlines	**SCR**	**D1**	**D2**	**D3**	**D4**	**D5**	**D8**	**D11**	**D 15**	**D 21**
Intervals (days)							**±1**	**±1**	**±1**	**±1**
Signed informed consent form	**X**									
Inclusion/exclusion criteria	**X**	**X**								
Assignment of the research participant number	**X**									
Demographic Data	**X**									
Medical background check		**X**								
Weight	**X**									
Vital signs and pulse oximetry		**X**								
Calculation of NEWS -2 score:		**X**								
Collection of clinical data		**X**								
Urine sample to evaluate pregnancy	**X**									

Blood Sample to evaluate Hepatic enzymes [2]	x [2]									
Randomization		x								
Dispensing and initiating medication/placebo		x								
Evaluation of the 7-point ordinal scale		x	x	x	x	x	x	x	x	x
Assessment of secondary outcomes - Pt. at home or hospitalized? - Has required oxygen? - Has required ICU? - Vital status - Persists with fever?		x	x			x	x	x	x	x
Collection of Serious Adverse Events (SAE)			x			x	x	x	x	x
Pharmacotherapeutic Adherence		x				x				
Remember the need to take the investigation product			x	x	x	x				
Collect symptoms reported to the admin of the investigation product			x	x	x	x	x	x	x	x
Record of completion										x

[1] In the case of hospitalized research participants, the study staff will personally visit the subject and/or contact the physician in charge by telephone, and/or through the medical history will obtain the necessary information and thus be able to determine primary or secondary outcomes

[2] It will not be necessary to collect the liver enzyme sample if there is a recent result no more than 3 days prior to selection.

4.3.4 Masking and blinding

Placebo will be administered to half of the research participants. The placebo will consist of a product with the same physical characteristics (taste, smell, texture, appearance) as ivermectin, but without the active ingredient. This placebo will be relabelled so that it looks the same as the investigation drug.

4.3.5 Follow up

To evaluate the evolution of the research participants and determine safety and efficacy according to the primary and secondary outcomes, subjects will be called on days 2, 3, 4, 5, 8, 11, 15 and 21 to fill out a questionnaire. In the event that the research participant requires hospitalization, the research participant will be visited personally to extract the information from the medical record. On the 21st day, the study will finish the evaluation of primary and secondary outcomes. In the case of hospitalized research participants, follow-up will continue until the end of hospitalization to determine their vital status.

4.3.6 Adherence to treatment

The administration of the research product will be done by self-administration in case of outpatient research participants, and in the case of hospitalized participants the administration instructions will be given to the subject and / or the health personnel who attends him/her, complying with the requirements of the institution.

Research participants will be contacted daily by phone call to remind them to take the research product, assess drug adherence, and detect adverse events to the research product.

4.3.7 Measurement of Security Parameters

On the day of the screening visit, all participants will have their liver enzymes measured. The research participant will not be randomized and therefore will not be included in the study if these are elevated> 1.5 times normal values.
In follow-up calls (days 2, 3, 4, 5, 8, 11, 15, and 21) ,research participants will be asked about symptoms that have historically been reported in subjects receiving ivermectin (uncontrollable shaking of a body part, dizziness, loss of appetite, nausea, vomiting, stomach pain or bloating, diarrhea, constipation, weakness, drowsiness, chest discomfort, swelling (face, arms, hands, feet, ankles, or lower legs), and other symptoms, pain and swelling in joints, painful and swollen glands in the neck, armpit, or groin, fast heartbeat, eye pain, blurred vision, redness or tearing, swelling (face, arms, hands, feet, ankles or calves), pain and swelling in the joints, painful and swollen glands in the neck, armpit or groin, fast heartbeat, pain in the eyes, blurred vision, redness or tearing, swelling of the eyes or eyelids, abnormal

sensation in the eyes, fever, blisters or peeling of skin, rash, hives, and itching) to complete the information requested by the data collection form.
The proportion of research participants with adverse events in both arms will be compared.

4.4 Sample size

According to the literature and previous data in this study, 75% of the subjects will have the event of interest (disappearance of symptoms) by day 21. Thus, including 390 subjects, we will identify 290 events of interest. With this sample size we will have a power of 80% and an Alpha of 0.05 to detect a Hazard ratio of 1.4 of ivermectin vs. placebo in time to symptom resolution. Taking into account a 2 % follow-up loss and 76 subjects who will be excluded from the primary analysis[1] , we will include a total of 476 subjects in the study.

4.5 Data analysis

An interim analysis will be conducted without breaking the blind when 200 research participants have been randomized. The study will be discontinued if this analysis detects a significant treatment effect on the main objective, if it is detected that a significantly larger group of subjects withdraw from the intervention arm due to adverse events, or if no difference is detected between the two groups. Recruitment will continue while this analysis is performed.

The information will be analyzed with Stata version 14 (SE; Stata corporation, College Station, Texas). For this purpose, subjects will be grouped into 2 groups that comprise

Group 1: Subjects who received ivermectin
Group 2: Subjects who received placebo.

The baseline characteristics of the subjects will be described by descriptive analysis.

The main analysis will be by intent-to-treat. The time until the disappearance of symptoms will be determined when all research subjects reach day 21. Clinical deterioration or death before the 21st will be right sensored- on the 21st. Deaths will be included unless there is strong evidence that the cause of death was not associated with COVID-19. The time until the disappearance of symptoms will be presented in a Kaplan-Meier curve and will be compared with the log-rank test. Hazard Ratios will be calculated with their 95% confidence interval through a Cox proportional-hazards model.

[1] 76 subjects included in the study had errors in the enlistment of the investigation product consecutively for three weeks, and will be excluded from the primary analysis.

Secondary outcomes and adverse events in each of the groups will be expressed by absolute and relative frequencies with their corresponding 95% confidence interval. For the univariate analysis in the quantitative variables, statistical parameters of central tendency and dispersion (means and standard deviations) will be described if the variable has a normal distribution. In case of not having a normal distribution, medians and interquartile ranges will be described. The qualitative variables will be measured and analyzed through proportions with their corresponding 95% confidence interval.

To determine the efficacy of the intervention in secondary outcomes, a bivariate analysis will be performed, evaluating the association of treatment, demographic and clinical variables with the different outcomes.

5. ETHICAL CONSIDERATIONS

5.1. Ethical conduct of the study

The conduct of this study will be in accordance with all local and/or national regulations and directives.

In accordance with international guidelines, this study is considered to comply with the following principles to protect research subjects:

1. Social and clinical value: SARS CoV2 is so far a virus with high rates of morbidity and mortality, but without specific treatment. Demonstrating the effectiveness of a safe treatment in the early stages could mitigate the negative impact that this pathology currently has.

2. Scientific validity: There is no therapy so far that has been proven to be effective for this pathology in its initial stages. Ivermectin has biological plausibility and an observational study in patients with SARS-CoV-2 suggests clinical utility.

3. Fair selection of research subjects: Individuals with a confirmed diagnosis of SARS CoV 2 are chosen. Randomization will guarantee a homogeneous distribution of confounders in both arms.

4. Risk-benefit ratio - favorable: Ivermectin is a safe drug, widely used in humans with different pathologies, so we do not anticipate adverse events. The potential benefit to subjects receiving the drug may be greater in mitigating SARS-CoV-2 disease. All subjects will be contributing to the development of science at this time of global emergency, by providing information aimed at improving the management of this disease.

5. Informed consent: All subjects must accept their participation in the study by signing an informed consent form and can withdraw their participation at any time.

6. Participation in the study does not generate any economic benefit for the subject, no incentives will be given and since there is no mobilization, transportation and food expenses are not covered.

5.2. Source data and documents

The "source data" is the data contained in the source documents. Source documents are original documents or certified copies and include, but are not limited to, memory aids, medical and hospital records, pre-selection records, informed consent/assent forms, telephone contact records, and worksheets. The purpose of the study source documents is to document the existence of the subjects, and to support the integrity of the data collected in the study. Investigators should maintain the source documents of the study so that they are accurate and legible, and are complete and up-to-date.

The investigator and center staff handling source documents must follow good documentation practices.

5.3. Confidentiality of data and access to participant records

In the event that a subject's medical history is not at the research center, it is the responsibility of the investigator to obtain said history if necessary.

All personal data collected related to subjects, investigators or anyone involved in the study, which may be included in the sponsor's database, will be treated in accordance with all applicable laws and regulations. The data collected should be adequate, relevant and not excessive in relation to the purposes for which it is collected. Each category of data must be adequately justified and be in line with the objective of the study.

Subjects will be assigned a unique identifier by the investigator. Any subject record or data set that is transferred will contain only the identifier; the names of the subject or any information that could make the subject identifiable will not be transferred.

The subjects must be informed that their personal data related to the study will be used by the investigator, in accordance with local data protection laws. The level of disclosure should also be explained to the subject.

The subject must be informed that their medical history may be examined by Quality Management auditors or other authorized persons designated by the investigator, by members of CEI, and by inspectors from regulatory authorities.

When filing or processing personal data relevant to the investigator and/or subjects, all appropriate measures will be taken to safeguard and prevent access to this data by an unauthorized third party.

5.4. Audits and inspections

The Quality Management area or an independent auditor can carry out a quality assurance audit at any time, to verify that the study has been carried out following the protocol and other applicable regulations. Regulatory authorities may conduct an inspection. The investigator must allow direct access to study documents during these inspections and audits.

5.5. Archive

The investigator must keep all study documents after its completion or interruption, for as long as the applicable laws and regulations require. The study documents will be kept for at least 25 years. Study personnel must not destroy or allow the destruction of any study document without written notice.

Archived data may be kept in electronic records, as long as a backup copy is available, and a hard copy can be obtained upon request. The protocol, documentation, approvals, and all other documents related to the study will be kept by the investigator in the CEIP Archive. All documents and data will be made available to the relevant authorities upon request.

5.6. Financial contract and insurance coverage

All parties involved in the conduct of the study will sign a study agreement, if applicable. CEIP has an insurance policy that covers any liability that may arise from the use of the study protocol.

5.7. Publishing policy

The data obtained from this study are the exclusive property of CEIP. Any publication or presentation related to the study should be sent to CEIP for review before the manuscript is submitted. After publication of study results, any participating center may publish or otherwise use its own data, as long as any publication of study data acknowledges the study group. In addition, CEIP will be offered association with all publications of this nature, with the understanding that CEIP has the right to reject such association.

6. EXPECTED RESULTS

After this study, we hope to understand the efficacy and safety of ivermectin for the treatment of adult patients with mild SARS CoV 2 disease.

7. BIBLIOGRAPHY

1. Corman VM, Lienau J, Witzenrath M. [Coronaviruses as the cause of respiratory infections]. *Internist (Berl).* 2019;60(11):1136-1145.

2. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol.* 2016;14(8):523-534.
3. Fehr AR, Channappanavar R, Perlman S. Middle East Respiratory Syndrome: Emergence of a Pathogenic Human Coronavirus. *Annu Rev Med.* 2017;68:387-399.
4. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497-506.
5. 2020; https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6. Accessed April 25, 2020.
6. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet.* 2020;395(10224):565-574.
7. Zhang J, Litvinova M, Wang W, et al. Evolving epidemiology and transmission dynamics of coronavirus disease 2019 outside Hubei province, China: a descriptive and modelling study. *Lancet Infect Dis.* 2020.
8. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal.* 2020.
9. Velavan TP, Meyer CG. The COVID-19 epidemic. *Trop Med Int Health.* 2020;25(3):278-280.
10. Departamental SdS. Comunicación Personal. 2020.
11. C.A S. Consenso colombiano de atencion, diagnóstico y manejo de la infección por SARS-CoV-2/COVID-19 en establecimientos de atención de la salud. *INFECTIO.* 2020;24.
12. Uyeki TM, Bernstein HH, Bradley JS, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenzaa. *Clin Infect Dis.* 2019;68(6):895-902.
13. King CL. Is ivermectin safe in pregnancy? *Lancet Glob Health.* 2020;8(1):e12-e13.
14. Campbell WC. *Ivermectin and Abamectin.* 1989.
15. Omura S. Ivermectin: 25 years and still going strong. *Int J Antimicrob Agents.* 2008;31(2):91-98.
16. Crump A, Omura S. Ivermectin, 'wonder drug' from Japan: the human use perspective. *Proc Jpn Acad Ser B Phys Biol Sci.* 2011;87(2):13-28.
17. Yang SN, Atkinson SC, C. W, al. e. The broad spectrum antiviral ivermectin targets the host nuclear transport importin α/β heterodime. *Antiviral research.* 2020.
18. Wagstaff KM, Sivakumaran H, Heaton SM, Harrich D, Jans DA. Ivermectin is a specific inhibitor of importin alpha/beta-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochem J.* 2012;443(3):851-856.
19. Mastrangelo E, Pezzullo M, De Burghgraeve T, et al. Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. *J Antimicrob Chemother.* 2012;67(8):1884-1894.
20. Tay MY, Fraser JE, Chan WK, et al. Nuclear localization of dengue virus (DENV) 1-4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin. *Antiviral Res.* 2013;99(3):301-306.
21. Lundberg L, Pinkham C, Baer A, et al. Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan Equine Encephalitis Virus replication. *Antiviral Res.* 2013;100(3):662-672.

22. Atkinson SC, Audsley MD, Lieu KG, et al. Recognition by host nuclear transport proteins drives disorder-to-order transition in Hendra virus V. *Sci Rep.* 2018;8(1):358.
23. Shechter S, Thomas DR, Lundberg L, et al. Novel inhibitors targeting Venezuelan equine encephalitis virus capsid protein identified using In Silico Structure-Based-Drug-Design. *Sci Rep.* 2017;7(1):17705.
24. Caly L, Wagstaff KM, Jans DA. Nuclear trafficking of proteins from RNA viruses: potential target for antivirals? *Antiviral Res.* 2012;95(3):202-206.
25. Jans DA, Martin AJ, Wagstaff KM. Inhibitors of nuclear transport. *Curr Opin Cell Biol.* 2019;58:50-60.
26. E Y, P A, D M. Efficacy and Safety of Ivermectin against Dengue Infection: A Phase III, Randomized, Double blind, Placebo-controlled Trial. Paper presented at: The 34th Annual Meeting The Royal College of Physicians of Thailand; 26th - 28th April 2018,, 2018; PEACH Royal Cliff Beach Resort, Pattaya, Chonburi, Thailand.
27. L C, J D, M C, al. e. The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Research.* 2020.
28. A. P, S. D, D. G, MR M. Usefulness of Ivermectin in COVID-19 Illness. 2020.
29. Steel C, Lujan-Trangay A, Gonzalez-Peralta C, Zea-Flores G, Nutman TB. Immunologic responses to repeated ivermectin treatment in patients with onchocerciasis. *J Infect Dis.* 1991;164(3):581-587.
30. Knab J, Darge K, Buttner DW. Immunohistological studies on macrophages in lymph nodes of onchocerciasis patients after treatment with ivermectin. *Trop Med Int Health.* 1997;2(12):1156-1169.
31. Soboslay PT, Dreweck CM, Hoffmann WH, et al. Ivermectin-facilitated immunity in onchocerciasis. Reversal of lymphocytopenia, cellular anergy and deficient cytokine production after single treatment. *Clin Exp Immunol.* 1992;89(3):407-413.
32. Akuffo H, Maasho K, Lavebratt C, Engstrom K, Britton S. Ivermectin-induced immunopotentiation in onchocerciasis: recognition of selected antigens following a single dose of ivermectin. *Clin Exp Immunol.* 1996;103(2):244-252.
33. Semnani RT, Keiser PB, Coulibaly YI, et al. Filaria-induced monocyte dysfunction and its reversal following treatment. *Infect Immun.* 2006;74(8):4409-4417.
34. Dobson RJ, Besier RB, Barnes EH, et al. Principles for the use of macrocyclic lactones to minimise selection for resistance. *Aust Vet J.* 2001;79(11):756-761.
35. Costa JL, Diazgranados JA. Ivermectin for spasticity in spinal-cord injury. *Lancet.* 1994;343(8899):739.
36. Diazgranados JA, Costa JL. Deaths after ivermectin treatment. *Lancet.* 1997;349(9066):1698.
37. J D, J M-F, L C-G. Ivermectina como coadyuvante en la epilepsia refractaria. *Rev Neurol.* 2017;65:303-310.
38. Diazgranados-Sanchez JA. [Potential interactions with ivermectin as adjuvant therapy for refractory epilepsy. Reply]. *Rev Neurol.* 2018;66(7):251-252.
39. Diazgranados-Sanchez JA, Barrios-Arrazola G, Costa JL, Burbano-Pabon J, Pinzon-Bedoya J. [Ivermectin as a therapeutic alternative in neurocysticercosis that is resistant to conventional pharmacological treatment]. *Rev Neurol.* 2008;46(11):671-674.
40. Smit MR, Ochomo EO, Aljayyoussi G, et al. Safety and mosquitocidal efficacy of high-dose ivermectin when co-administered with dihydroartemisinin-piperaquine in

Summary of Changes in the Protocol

		JUSTIFICATION		
Amendment 2 to the Study Protocol_August 04, 2020.	a. The title was changed: The study was extended to other municipalities in Valle del Cauca near the capital. B. Included: the use of SARS-CoV-2 antigen detection in nasopharyngeal sample. C. A secondary objective was added: the duration of fever as an indirect marker of the antiviral capacity of the drug. d. Placebo produced by Tecnoquimicas laboratory was included. e. Changed: Onset of SARS CoV2 / COVID 19 illness 7 days ago or less. f. Liver tests taken within 3 days were included. g. The range of liver enzymes was increased > 1.5 h. Order of procedures was corrected	1. Due to the difficulty in identifying subjects in the city of Cali, we will extend the study to other municipalities in Valle del Cauca near the capital. 2. Due to the congestion of health services due to COVID-19 and the high demand for testing, the opportunity for RT-PCR reporting is scarce and the results of a patient are usually available after 5 days of taking it. In May 2020 and August 2020, the FDA (United States) and the Ministry of Health of Colombia, respectively, have approved the use of antigen detection as valid diagnostic tests for the diagnosis of COVID-19. Through this amendment we intend to identify subjects in the initial stages of the disease and improve the recruitment of subjects, this is included in the inclusion criteria. 3. Duration of fever: This secondary objective is added as an indirect marker of the antiviral capacity of the drug. 4. The placebo was changed from 5% dextrose to placebo: in the process of elaboration of the protocol, the pharmaceutical laboratory that produces the investigation product did not confirm that it could produce the placebo with the same characteristics but without active ingredient. Only until now we have been informed that they can produce it, for this reason this final amendment 2 from July 17, 2020 is included and the Investigator's manual is updated. 5. Inclusion criteria: it is increased to 7 days, taking into account that during the elaboration of the protocol until now, it is very difficult to obtain the tests results within 5 days due to high demand in the laboratories that process the samples. By broadening this selection criterion, the objective of making an intervention in the earliest stage of the disease is maintained, allowing us to reach the proposed sample size. 6. Exclusion criterion: The increase in the elevation range from 1.25 to 1.5 times is generated because we are having a large number of subjects with screening failures due to minimal elevations in liver enzymes. These elevations are usually transitory and produced by conditions other than liver pathologies (produced by puncture, injections, mild trauma, stress from acute infection, use of other medications, fatty foods, laboratory errors, etc.). For this reason, increasing the elevation range from 1.25 to 1.5 times does not increase risks for the subject when entering the study. 7. Selection criterion: The range of liver enzymes was increased > 1.5: We found that some subjects may have recent reliable liver enzyme test results within the established time frame (days prior to screening). We can decrease the risk involved in a puncture (bruising, infection, etc.) and yet we can verify the exclusion criteria indicated in the protocol with these recent tests. 8. Exclusion criterion: Patients with progressive improvement of symptoms: This criterion is added taking into account that we extended the inclusion criterion to 7 days. However, subjects who present progressive improvement between days 5-7 are at low risk of clinical deterioration. Thus their inclusion would reduce the power of the study. 9. Table of visits and procedures: the name of visits is changed. This change is justified in that they can be carried out on the same day.	New ICF issued Informed Consent Format and General Informed Consent of the study, Version 5.0 of August 04, 2020.	**Approved by INVIMA on August 27, 2020:**
Final Amendment 3 to the Study Protocol_September 16, 2020	a. This specific objective was included: "To compare the time between randomization and disappearance of symptoms in subjects receiving the D11AX22 molecule (ivermectin) vs. Placebo" b. The original primary outcome was changed to: "To determine the time until the resolution of the symptoms in the two study groups." c. The original primary outcome becomes a secondary outcome	Less than 3% of the subjects included to date have had the primary outcome described in Amendment 2 ("To determine the time to deterioration in two or more points of the 7-point ordinal scale in the two study groups."). If this primary outcome were retained, the sample size to obtain adequate statistical power would be unattainable (more than 2000 subjects). With this amendment we intend to meet the general and specific objectives with the initially proposed sample size.	No new ICF issued	**Approved by INVIMA on November 06, 2020**
Final Amendment 4 to the Study Protocol_October 27, 2020	a. Sample size increased to 476	After the start of the study, 38 subjects who were randomized to receive placebo were given ivermectin by mistake in the enrollment of the investigation product. This occurred consecutively over a period of three weeks, so during this period a total of 76 subjects received ivermectin and none received a placebo. This situation was discussed with the independent data monitoring board. Taking into account that this is a systematic error, in which none of these participants received the randomly assigned investigation product, the exclusion of these 76 subjects from the primary analysis was decided. To preserve the planned sample of 400 subjects and thus preserve the statistical power of the study, an additional 76 participants will be included for a total of 476 participants. The 76 participants excluded from the primary analysis will complete the follow-up established by the protocol.	INFORMED CONSENT FORM AND GENERAL INFORMED CONSENT OF THE STUDY_Version 6.0_October 27, 2020	**Approved by INVIMA on November 9, 2020:**

Statistical Analysis Plan

(SAP)

Protocol Tittle	Double blind, randomized, controlled clinical trial to investigate the effectiveness of the d11ax22 molecule in adult subjects from Valle del Cauca with initial stages of infection by SARS-CoV-2/ Covid-19.
Acronym	EPIC
Protocol version	Final Version 2.0: May 12, 2020
ClinicalTrials.gov	NCT04405843
Principal Investigator	Dr. Eduardo López, MD, MSc Centro de Estudios en Infectología Pediátrica Universidad del Valle Clinica Imbanaco
Author	Erika Cantor, BSc, MSc
Version	Jun 7, 2020

Abbreviations

ECC	Controlled Clinical Trial
ICU	Intensive Care Unit
ITT	Intension to treat
PP	Per protocol

Table of contents

1. Introduction

The purpose of this study is to assess whether D11AX22 molecule (ivermectin) reduces the time until worsening in 2 or more points in the 7-point ordinal scale (primary outcome variable), as well as the proportion of patients who require hospitalization, use of supplementary oxygen and intensive care (secondary outcome variables), compared to placebo in subjects with mild COVID-19 disease. This document describes definitions and statistical methods to be used in order to fulfill EPIC trial aim and objectives.

2. Study Design

Double-blind, randomized, placebo-controlled clinical trial to evaluate the efficacy of the D11AX22 (ivermectin) molecule in adult subjects with mild SARS CoV2/COVID-19 disease at participating institutions in Valle del Cauca.

This study is a controlled clinical trial with two parallel group with an allocation ratio of 1:1. Ten clinical visits will be made at the following times: day 0 (visit 1), day 1 (visit 2) day 2, day 3, day 4, day 5, day 8, day 11, day 15 and day 21.

2.1. Interventions

Intervention group or treatment: Participants assigned to **ivermectin**, once a day for 5 days.

Treatment name: Molecule D11AX22 (ivermectin)
Drug name: ivermectin
Drug presentation: oral drops in suspension 6 mg / ml.
Route of administration: oral
Primary container: 5mL bottle
Volume to be administered: depends on the patient's weight
Dose: 300 mcg/kg/day
Storage conditions on the label: room temperature
Indications for administration: preferably a single dose on an empty stomach, with water.
Serious interactions: Warfarin, erdafitinib, quinidine

Control Group: Participants assigned to **placebo**, once a day for five days.

Treatment name: placebo

Drug Name: 5% Dextrose

Drug Presentation: solution

Route of administration: oral

Primary container: 5mL bottle

Volume to be administered: depends on the patient's weight

Dose: same volume that would be administered of ivermectin to maintain the blind

Storage conditions on the label: room temperature

Indications for administration: same as ivermectin.

2.2. Sample size calculation

According to the literature, 20% of patients will develop the primary outcome (worsening of 2 or more points in the 7-point ordinal scale). Thus, we will need to include 400 patients (72 total events plus 10% lost events) in order to detect a Hazard ratio of 0.5 of ivermectin vs. placebo in time to deterioration, with a power of 80% and alpha of 0.05

3. Study Aim

To describe the efficacy of the D11AX22 (ivermectin) molecule in adults with mild COVID-19 disease

4. Outcomes

4.1. Primary outcome

-Time until clinical worsening in the two study groups: Number of days from randomization visit to the first day when a patient worsens 2 or more points in the 7-point ordinal scale in the two study groups. Variable measured during the 21 days of follow-up.

4.2. Secondary outcomes

-Proportion of cases that require hospitalization, use of supplemental oxygen> 24 hours, and admission to the ICU.

-Duration of hospitalization, use of supplemental oxygen and duration of stay in ICU in each arm of the study.

-Clinical state, using the 8-point ordinal scale, on days 2, 5, 8, 11, 15, and 21.

4.3. Safety outcomes

-Proportion of subjects who develop adverse events and serious adverse events associated with the study drug: patients who during follow-up report episodes of uncontrollable tremor, dizziness, loss of appetite, nausea, vomiting, stomach pain, bloating, diarrhea, constipation, weakness, drowsiness, chest discomfort, edema (face, arms, hands, feet, ankles or calves), pain and swelling in the joints, painful and swollen glands in the neck, armpit or groin, fast heartbeat, eye pain, blurred vision, redness or tearing, swelling of the eyes or the eyelids, abnormal sensation in the eyes, fever, blistering or peeling of the skin, rash, hives, and itching.

-Proportion of subjects who required discontinuation of medication due to adverse events.

5. Study population and subgroup analysis

5.1. Populations

Intention to Treat (ITT). It is the primary population of the EPIC trial and includes all patients randomized in the study. They will be analyzed according to the group assigned at the randomization visit, regardless of whether they received the medication or not.

Per protocol (PP). All patients who were adherent to the assigned intervention at the time of randomization. In addition, it includes all patients with complete data. It will be considered as a sensitivity analysis.

5.2. Subgroups

No subgroup analysis will be done.

6. Analysis plan

Initially, all the variables will be described according to the intervention groups in the ITT population. Variables with a normal distribution will be summarized with mean and standard deviation (SD), otherwise, they will be shown with median and interquartile range (IQR). Normal distribution assumption will be evaluated using the Shapiro Wilk test. All analyzes will be done in Stata 14.0® (StataCorp, College Station, Texas, USA).

6.1. Primary outcome

The main analysis will be the survival analysis for the primary outcome variable (time to deterioration in 2 or more points in the 7-point ordinal scale) using the log-rank test to compare ivermectin versus placebo as a control group. Deterioration or worsening will be assessed using the 7-point ordinal scale, which has the following categories: (1) -Non-hospitalized patient, with the ability to do daily activities, (2) -Non-hospitalized patient, unable to do daily activities, or using oxygen at home (3) -Hospitalized, without need for supplemental oxygen, (4) -Hospitalized, with need for supplemental oxygen, (5) -Hospitalized, with need for high-flow nasal oxygen or non-invasive mechanical ventilation, (6) -Hospitalized, in need of mechanical ventilation or ECMO, (7) -Death.
The outcome variable will be the time to worsening, defined as the first day, during the 21 days of follow-up, in which the patient reports an increase of 2 or more points in the ordinal scale as compared with the baseline score when randomized.

Patients who do not report deterioration in 2 or more points and those who die during study follow-up will be considered as censor data on day 21 or until death. Kaplan-Meier estimator will be used to report the recovery percentage throughout the follow-up and at

day 21. Relevant treatment efficacy parameter is the recovery hazard ratio (HR) with its respective 95% confidence interval (CI), which will be estimated by Cox proportional-hazard model. Given that the primary outcome may take two values; equal to 1 when the patient reports deterioration and 0 when not, a HR< 1 will indicate a favorable result for ivermectin compared to placebo.

6.2. Secondary outcomes

Secondary outcomes, hospitalization requirement, ICU or oxygen supplementation will be analyzed through a Kaplan Meier estimate. Hazard ratio will be estimated by Cox proportional-hazard model. In addition, a logistic regression model in which each secondary outcome will be considered as the dependent variable (1: Event, 0: No event) and the intervention groups as the independent variable (1: ivermectin, 0: Placebo) will be used.

For each outcome, the odds ratio (OR) and its respective 95% CI will be reported. An OR> 1 is indicating favorable results in favor of placebo and an OR <1 in favor of ivermectin. Evaluation of the effect of the treatment in each study visit using the 7-point ordinal scale will be estimated using the proportional OR with its respective 95% CI with an ordinal logistic regression. In this step, the 7-point ordinal scale will be inverted in its score, where 1 will correspond to death and 7 to a subject without symptoms. Thus, a proportional OR> 1 will indicate an effect in favor of ivermectin. The 95% confidence interval of the median difference in length of hospitalization or oxygen use will be estimated by bootstrap estimators.

To further determine the efficacy of the intervention in secondary outcomes, a bivariate analysis will be performed adjusting for demographic and clinical variables. For dichotomous variables, a logistic regression will be performed and the adjusted odds ratio will be reported. For time to event variables, an adjusted cox regression model will be performed and the hazard ration will be reported. In either case, the measure of effect will be reported with its respective 95% CI.

6.3. Sensitivity Analysis and Modification of the Effect for the Primary outcome.

The analysis of the primary outcome will be repeated using the population under the per protocol approach and will be considered as sensitivity analysis. Additionally, if differences are found between the research product and the outcomes, it will be evaluated whether variables such as enrollment score on the 7-point scale, duration of symptoms before enrollment, comorbidities, age and sex, modify the effect of the intervention groups, using a multivariate Cox proportional hazards model. This will be done by including interaction terms to the model between the possible modifying variable and the intervention groups. A p value <0.10 will be considered as a possible modifying effect, and a p value <0.05 will be considered as a cut-off point to confirm a significant interaction and therefore, to estimate the effect of the treatment groups with their respective 95% CI.

6.4. Safety analysis

This analysis will be carried out on an exploratory basis and the frequency and detailed description of each adverse event will be reported.

6.5. Handling missing data

Primary outcome:

The primary outcome is expected to be available in all patients. In the event that the patient dies during follow-up, a censoring for the study will be considered until the time of last observation. If the patient is lost to follow-up, last report on the 7-point scale during follow-up will be considered as the outcome.

Covariates: If there are missing data in the variables needed to analyze for effect modification, and this may have an effect the primary outcomes of the study, then simple imputation will be performed using the median of the treatment group to which the observation belongs based on the information reported in the sample with complete data.

Secondary outcomes. Data will not be imputed. The percentage of missing data will be reported.

Statistical Analysis Plan (SAP)

Protocol Tittle	Double blind, randomized, controlled clinical trial to investigate the effectiveness of the d11ax22 molecule in adult subjects from Valle del Cauca with initial stages of infection by SARS-CoV-2/ Covid-19.
Acronym	EPIC
Protocol version	Final amendment 3, September 16, 2020
ClinicalTrials.gov	NCT04405843
Principal Investigator	Dr. Eduardo López, MD, MSc Centro de Estudios en Infectología Pediátrica Universidad del Valle Clinica Imbanaco
Author	Erika Cantor, BSc, MSc
Version	PAE 1.0, Sep 20 2020

Abbreviations

ECC	Controlled Clinical Trial
ICU	Intensive Care Unit
ITT	Intension to treat
PP	Per protocol

Table of contents

1. Introduction

The purpose of this study is to assess whether D11AX22 molecule (ivermectin) reduces the time to resolution of symptoms (primary outcome variable), as well as the proportion of hospitalization, use of supplementary oxygen and number of cases with fever or more than two points on the eight-point ordinal scale for COVID (secondary outcome variables), compared to placebo in subjects with mild COVID-19 disease. This document describes definitions and statistical methods to be used in order to fulfill EPIC trial aim and objectives.

2. Study Design

Double-blind, randomized, placebo-controlled clinical trial to evaluate the efficacy of the D11AX22 (ivermectin) molecule in adult subjects with mild SARS CoV2/COVID-19 disease at participating institutions in Valle del Cauca.

This study is a controlled clinical trial with two parallel group with an allocation ratio of 1:1. Ten clinical visits will be made at the following times: screening visit, randomization visit, day 2, day 3, day 4, day 5, day 8, day 11, day 15 and day 21.

2.1. Interventions

Intervention group or treatment: Participants assigned to **ivermectin**, once a day for 5 days.

Treatment name: Molecule D11AX22 (ivermectin)

Drug name: ivermectin

Drug presentation: oral drops in suspension 6 mg / ml.

Route of administration: oral

Primary container: 5mL bottle

Volume to be administered: depends on the patient's weight

Dose: 300 mcg/kg/day

Storage conditions on the label: room temperature

Indications for administration: preferably a single dose on an empty stomach, with water.

Serious interactions: Warfarin, erdafitinib, quinidine

Control Group: Participants assigned to **placebo**, once a day for five days.

Treatment name: placebo

Drug Name: placebo

Drug Presentation: solution

Route of administration: oral

Primary container: 5mL bottle

Volume to be administered: depends on the patient's weight

Dose: same volume that would be administered of ivermectin to maintain the blind

Storage conditions on the label: room temperature

Indications for administration: same as ivermectin.

2.2. Sample size calculation

According to the literature and previous data in this study, 75% of the subjects will have the event of interest (disappearance of symptoms) by day 21. Thus, by including 390 subjects, we will identify 290 events of interest. With this sample size we will have a power of 80% and an Alpha of 0.05 to detect a Hazard ratio of 1.4 of ivermectin vs. placebo in time to symptom resolution. Taking into account a 2% follow-up loss and 76 subjects who will be excluded from the primary analysis, we will include a total of 476 subjects in the study.

3. Study Aim

To describe the efficacy of the D11AX22 (ivermectin) molecule in adults with mild COVID-19 disease

4. Outcomes

4.1. Primary outcome

-Time until the resolution of the symptoms in the two study groups. Number of days from randomization visit to the first symptom-free day (score of 0 on the 8-point scale). Variable measured during the 21 days of follow-up.

4.2. Secondary outcomes

-Proportion of cases with deterioration in two or more points of the 8-point ordinal scale in the two study groups. Proportion of subject who reported an increase in disease severity of 2 or more points on the 8-point ordinal scale relative to the randomization visit.

-Proportion of cases that required hospitalization, use of supplemental oxygen> 24 hours, and admission to the ICU.

-Duration of hospitalization, use of supplemental oxygen and duration of stay in ICU in each arm of the study.

-Clinical state, using the 8-point ordinal scale, on days 2, 5, 8, 11, 15, and 21.

-Proportion of cases who develop fever (axillary temperature> 38C or subjective feeling of fever) in each arm of the study and duration of fever. Number of days from the randomization visit to an axillary temperature <38ºC.

4.3. Safety outcomes

-Proportion of subjects who develop adverse events and serious adverse events associated with the study drug: patients who during follow-up report episodes of uncontrollable

tremor, dizziness, loss of appetite, nausea, vomiting, stomach pain, bloating, diarrhea, constipation, weakness, drowsiness, chest discomfort, edema (face, arms, hands, feet, ankles or calves), pain and swelling in the joints, painful and swollen glands in the neck, armpit or groin, fast heartbeat, eye pain, blurred vision, redness or tearing, swelling of the eyes or the eyelids, abnormal sensation in the eyes, fever, blistering or peeling of the skin, rash, hives, and itching.

-Proportion of subjects who required discontinuation of medication due to adverse events.

5. Study population and subgroup analysis

5.1. Populations

Intention to Treat (ITT). It is the primary population of the EPIC trial and includes all patients randomized in the study. They will be analyzed according to the group assigned at the randomization visit, regardless of whether they received the medication or not.

Per protocol (PP). All patients who were adherent to the assigned intervention at the time of randomization. In addition, it includes all patients with complete data. It will be considered as a sensitivity analysis.

5.2. Subgroups

No subgroup analysis will be done.

6. Analysis plan

Initially, all the variables will be described according to the intervention groups in the ITT population. Variables with a normal distribution will be summarized with mean and standard deviation (SD), otherwise, they will be shown with median and interquartile range (IQR). Normal distribution assumption will be evaluated using the Shapiro Wilk test. All analyzes will be done in Stata 16.0® (StataCorp, College Station, Texas, USA).

6.1. Primary outcome

The main analysis will be the survival analysis for the primary outcome variable (time to disappearance of symptoms associated with COVID-19), using the log-rank test to compare ivermectin versus placebo as a control group. Symptom resolution will be assessed using the 8-point ordinal scale, which has the following categories: (0) -Patient without symptoms, (1) -Non-hospitalized patient, with the ability to do daily activities, (2) -Non-hospitalized patient, unable to do daily activities, or using oxygen at home (3) -Hospitalized, without need for supplemental oxygen, (4) -Hospitalized, with need for supplemental oxygen, (5) -Hospitalized, with need for high-flow nasal oxygen or non-invasive mechanical ventilation, (6) -Hospitalized, in need of mechanical ventilation or ECMO, (7) -Death.
The outcome variable will be the time to recovery, defined as the first day, during the 21 days of follow-up, in which the patient reports a score of (0) on the 8-point scale.

Patients who do not report recovery from symptoms and those who die during study follow-up will be considered as censor data on day 21 or until death. Kaplan-Meier estimator will be used to report the recovery percentage throughout the follow-up and at day 21. Relevant treatment efficacy parameter is the recovery rate ratio (RR) with its respective 95% confidence interval (CI), which will be estimated by Cox proportional-hazard model. Given that the primary outcome may take two values; equal to 1 when the patient reports recovery of symptoms and 0 when not, a RR> 1 will indicate a favorable result for ivermectin compared to placebo.

6.2. Secondary outcomes

Secondary outcomes, hospitalization requirement, ICU or oxygen supplementation and deterioration in more than two points on the scale of clinical results will be analyzed through a Kaplan Meier estimate. Hazard ratio will be estimated by Cox proportional-hazard model. In addition, a logistic regression model in which each secondary outcome will be considered as the dependent variable (1: Event, 0: No event) and the intervention groups as the independent variable (1: ivermectin, 0: Placebo) will be used.

For each outcome, the odds ratio (OR) and its respective 95% CI will be reported. An OR> 1 is indicating favorable results in favor of placebo and an OR <1 in favor of ivermectin. In case of low frequency of individual outcomes, a combined variable will be created using need for hospitalization (general floor or ICU) and need for oxygen (by nasal cannula or mechanical ventilation) and will be analyzed using a logistic regression model reporting the OR with its 95% CI.

Evaluation of the effect of the treatment in each study visit using the 8-point ordinal scale will be estimated using the proportional OR with its respective 95% CI with an ordinal logistic regression. In this step, the 8-point ordinal scale will be inverted in its score, where 0 will correspond to death and 7 to a subject without symptoms. Thus, a proportional OR> 1 will indicate an effect in favor of ivermectin. The 95% confidence interval of the median difference in duration of fever and length of hospitalization or oxygen use will be estimated by bootstrap estimators.

6.3. Sensitivity Analysis and Modification of the Effect for the Primary outcome.

The analysis of the primary outcome will be repeated using the population under the per protocol approach and will be considered as sensitivity analysis. Additionally, if differences are found between the research product and the outcomes, it will be evaluated whether variables such as enrollment score on the 8-point scale, duration of symptoms before enrollment, comorbidities, age and sex, modify the effect of the intervention groups, using a multivariate Cox proportional hazards model. This will be done by including interaction terms to the model between the possible modifying variable and the intervention groups. A p value <0.10 will be considered as a possible modifying effect, and a p value <0.05 will be considered as a cut-off point to confirm a significant interaction and therefore, to estimate the effect of the treatment groups with their respective 95% CI.

6.4. Safety analysis

This analysis will be carried out on an exploratory basis and the frequency and detailed description of each adverse event will be reported.

6.5. Handling missing data

Primary outcome:

The primary outcome is expected to be available in all patients. In the event that the patient dies during follow-up, a censoring for the study will be considered until the time of last observation. If the patient is lost to follow-up, last report on the 8-point scale during follow-up will be considered as the outcome.

Covariates: If there are missing data in the variables needed to analyze for effect modification, and this may have an effect the primary outcomes of the study, then simple imputation will be performed using the median of the treatment group to which the observation belongs based on the information reported in the sample with complete data.

Secondary outcomes. Data will not be imputed. The percentage of missing data will be reported.

Summary of Changes in the Statistical Analysis Plan

1. Introduction:
The original primary outcome (time until worsening in 2 or more points in the 7-point ordinal scale) was replaced by the final primary outcome (time to resolution of symptoms)

2.2 Sample size calculation:
The original sample size was calculated to 400, in order to detect a hazard ratio in time to deterioration of 0.5 of ivermectin vs. placebo. The sample size remained 400 patients, after the primary endpoint was modified. However, the sample size increased to 476 due to 76 patients who were excluded from primary analysis (76 subjects who had errors in the label of the investigation product consecutively for three weeks)

4.1 primary outcome
The primary outcome was changed to Time until resolution of symptoms in the two study groups

4.2 secondary outcomes
The primary outcome became a secondary outcome. The ordinal scale changed from 7 to 8 points to include the score of 0.

The proportion of subjects who develop fever was added as an indirect marker of the antiviral capacity of the drug

6. Analysis Plan
The STATA version was updated to 16.0

6.1 Primary outcome
The primary outcome was modified from time until deterioration to time until complete resolution of symptoms

The treatment efficacy parameter changed from the hazard ratio to the recovery rate ratio (for ivermectin relative to placebo), which is akin to the hazard ratio in survival analysis but for the beneficial outcome of recovery.

6.2 Secondary outcomes
A combined variable was created due to the low frequency of individual variables (hospitalization (general ward or ICU) and need for supplementary oxygen).

The bivariate analysis adjusting for demographic and clinical variables was removed as the randomization would provide balanced arms in terms of measured clinical and demographic variables.