Phase II randomized controlled trial to evaluate the efficacy and safety of HeberFERON versus Heberon alpha R in symptomatic or asymptomatic patients infected with the SARS-CoV-2 (Study ESPERANZA/HOPE).

Bello-Rivero Iraldo (iraldo.bello@cigb.edu.cu)
CIGB
Francisco Hernandez-Bernal
CIGB
Hugo Nodarse-Cuni
CIGB
Yaquelin Duncan-Roberts
CIGB
Claudia Martínez Suarez
CIGB
Ivan Campa-Legrá
CIGB
Idelsis Esquivel-Moynelo
Hospital Militar Central Luis Dia Soto
Verena Muzio-Gonzalez
CIGB
Gerardo Guillen-Nieto
CIGB

Study protocol

Keywords: SARS-Cov-2, Interferons, Cuba, Randomised controlled trial

Posted Date: December 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-28958/v2

Abstract

Background: As the outbreak of COVID-19 has accelerated, an urgent need for finding strategies to combat the virus is growing. Results from in vitro studies suggest that a combination of IFN type I and Type II may be effective against SARS-CoV. The aim of this study is to investigate the efficacy of treatment with a recombinant IFN alpha 2b and gamma, provided with standard protocol (Kaletra (lopinavir-ritonavir 200/50 mg; 200/100 mg every 12 hour for 30 days; Chloroquine (250 mg) every 12 hours for 10 days) for COVID-19 patients, compared to standard protocol (IFN alpha 2b/Kaletra/Chloroquine) for COVID-19 hospitalized patients, positive diagnosed for SARS-Cov-2.

Methods: Hospitalized adult patients with qPCR confirmed SARS-Cov-2 will be enrolled in this open-labeled, single center, prospective, randomized and controlled clinical trial. One hundred and twenty eligible patients with confirmed SARS-CoV-2 positivity by qPCR amplification in oropharyngeal/nasopharyngeal swab samples will be enrolled at “Luis Diaz Soto” Hospital, Havana, Cuba. The primary outcomes are the time to 2019-nCoV RNA negativity in patients and the time until progression to severe COVID-19.

Discussion: This will be the first randomized controlled trial of a potential treatment for SARC-Cov-2 using the combinations of IFNs.

Trial registration: The study is sponsored by Center for Genetic and Biotechnology and Ministry of Health of Cuba and was duly registered April 2020 at http://registroclinico.sld.cu/en/trials/RPCEC00000307-En. Enrolment for this study began in April 11, 2020, and has enrolled one hundred patients as of May-26-2020.

Administrative Information

Title {1}	Phase II randomized controlled trial to evaluate the efficacy and safety of HeberFERON versus Heberon alpha R in symptomatic or asymptomatic patients infected with the SARS-CoV-2 (Study ESPERANZA/HOPE).
Trial registration {2a and 2b}.	Cuban Public Registry of Clinical Trials (RPCEC), RPCEC00000307 Date registration 14/04/2020 Web address http://rpcec.sld.cu/en/trials/RPCEC00000307-En
Protocol version {3}	Protocol version I, 01/04/2020 and IG/IAG/CV/2001
Funding {4}	CIGB support the investigational products, and qPCR, FACS and other laboratory determinations. Ministry of Health support all the patient care, hospitalization, and clinical determinations (hematological, biochemical, microbiology, RX, electrocardiogram). The funder played no part in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication
Author details {5a}	Iraldo Bello Rivero. Center for Genetic Engineering and Biotechnology, Ave 31 e/ 158 and 190, Cubanacan, Playa, Havana, Cuba. (: 53-7 2087421, 2087465, ext 109. E-mail address: iraldo.bello@cigb.edu.cu -Francisco Hernandez-Bernal. Center for Genetic Engineering and Biotechnology, Ave31e/158 and 190, Cubanacan, Playa, Havana, Cuba. (: 53-7 2087421, 2087465, ext 148. E-mail address: hernandez.bernal@cigb.edu.cu -Hugo Nodarse-Cuni: Center for Genetic Engineering and Biotechnology, Ave31e/158 and 190, Cubanacan, Playa, Havana, Cuba. (:53-7 2087421, 2087465, ext 107. E-mail address: hugo.nodarse@cigb.edu.cu -Yaquelin Duncan-Roberts: Center for Genetic Engineering and Biotechnology, Ave31e/158 and 190, Cubanacan, Playa, Havana, Cuba. (: 53-7 2087421, 2087465, ext 109 E-mail address: yaquelin.duncan@cigb.edu.cu -Claudia Martínez Suarez: Center for Genetic Engineering and Biotechnology, Ave31e/158 and 190, Cubanacan, Playa, Havana, Cuba. (: 53-7 2087421, 2087465, ext 109. E-mail address: claudia.martinez@cigb.edu.cu -Ivan Campa-Legrá: Center for Genetic Engineering and Biotechnology, Ave31e/158 and 190, Cubanacan, Playa, Havana, Cuba. (: 53-7 2087421, 2087465, ext 109. E-mail address: ivan.campa@cigb.edu.cu -Idelsis Esquivel-Moynelo: Hospital Militar Central “Dr. Luis Díaz Soto”, Ave Monumental 2 , C.P. 19130, Habana del Este, La Habana. ((53-7)-680000; 0057; 0577; E-mail: idelsisem@infomed.sld.cu -Verena Muzio-Gonzalez: Center for Genetic Engineering and Biotechnology, Ave31e/158 and 190, Cubanacan, Playa, Havana, Cuba. (: 53-7 72087379. E-mail:verena.muzio@cigb.edu.cu -Gerardo Guillen Nieto: Center for Genetic Engineering and Biotechnology, Ave31e/158 and 190, Cubanacan, Playa, Havana, Cuba. (: 53-7 27160 ext 1252. E-mail: gerardo.guillen@cigb.edu.cu
Name and contact information for the trial sponsor {5b}	Hector Santana Milian. MSc. Direction of Pharmaceutical Development at Center for Genetic Engineering and Biotechnology. Ave31e/158 and 190, Cubanacan, Playa, Havana, Cuba hector.santana@cigb.edu.cu
Role of sponsor {5c}	The study sponsor and funders has no roles in collection, management, analysis, and interpretation of data; or writing of the report. The study sponsor and funders has no roles in the decision to submit the report for publication.

Introduction

Background and rationale {6a}

As the SARS-Cov-2 infection has spread to more than 180 countries of the world and generated a significant number of deaths, more than 5% of lethality, with great social and economic consequences, it is very urgent to find and develop strategies to combat this new virus.

There are guides issued by expert committees of WHO, Singapore, South Korea and US institutions, that recommend the clinical use of interferon (IFN) for the treatment and prevention of COVID-19[1,2,3]. Countries like China and Spain have incorporated this medicine in their protocols and national clinical guidelines for the care of this type of patient[4,5].

There are three types of Interferons (IFNs): type I (IFN-a and IFN-b), type II (IFN-g) and type III (IFN-l)[6]. IFNs are proteins created by nature for the first line defense against pathogens (viruses, bacteria, parasites), a function demonstrated in a group of species at different levels of the evolution chain, confirming their protective role.

Infection with mammalian cell viruses incites the innate immune system to establish a first line of defense. IFNs play a key role in these events, as they activate the innate immune system and help shape adaptive immunity[7]. IFN-g is the main modulator in establishing the relationship between these two types of immune responses[8].

IFNs possess pleiotropic effects that overlap on various cellular functions. IFNs-a and b have a greater antiproliferative and antiviral effect and IFN-g a superior immunoregulatory activity[9,10].

IFNs-a and -g exercise their functions through different but related signaling pathways. Studies in animal models have shown that IFNs-a and -g are essential for antiviral defense and are functionally non-redundant[11].

Viruses have developed mechanisms to evade the functions of the IFN system by simulating proteins that intervene in the synthesis mechanisms of IFNs, and intracellular signaling to establish the protection mechanisms by these (IFN receptor and other signaling cascade proteins)[12,13].

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2 is a positive-sense, enveloped, single-stranded RNA β coronavirus similar to Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) virus. This type of virus is susceptible to the antiviral action of IFNs, but variations have been developed to interfere with this activity[14].

Viruses have developed mechanisms to evade the functions of the IFN system by simulating proteins that intervene in the synthesis mechanisms of IFNs, and intracellular signaling to establish the protection mechanisms (IFN receptor and other proteins of signaling cascades) [15,13].

SARS-Co, which emerged in 2003 and caused widespread human mortality, encodes at least five proteins that function as IFN antagonists[16,17,18,19], suppressing signaling and production components. These proteins are believed to be responsible for the pathogenesis of SARS-CoV.

The induction of IFNs plays a fundamental role in defending the body against CoV infections. Numerous studies have presented the effectiveness of direct administration of IFN to eliminate these viruses[20].

IFN-g has been reported to have an antiviral effect in coronavirus infection. This effect is described mediated by the recruitment of monocytes and T lymphocytes to the area of the infected cells and the production of IFN-g by them. In the absence of IFN-mediated signaling, infection leads to the death of infected animals[21].

The IFN-α e IFN-g combination in the infection of coronavirus

The combination of IFN-g and -λ has been described to synergistically inhibit the replication of SARS-CoV in vitro[22,23]. Larkin et al. [24] indicate that the combination of IFN-α and IFN-g generates strong synergistic antiviral activity. Another study demonstrates that co-administration of IFN-α and IFN-g causes hyperactivation of IRF-1 and STAT1, ultimately leading to a more robust antiviral response against viral replication[25]. The combination of type I IFN (IFN-α, β) with IFN-γ synergistically inhibits virus replication in vitro[22].

The effects of IFNs on the replication of SARS-CoV, showed that treatment of Vero E6 cells with 100 U / mL of IFN-β or IFN-g marginally reduced viral replication. However, treatment with IFN-β and IFN-g inhibited SARS- CoV plaque formation 30 times and replication 3000 times at 24 h, and> 1 x 10^5 times at 48 and 72 h after the infection. This result demonstrates the synergistic inhibition of SARS-CoV replication by IFN type I and IFN-g22.

The higher mortality rate from COVID-19 in elderly patients has been associated with a greater delay between the time of infection and the response of the immune system, caused mainly by the inhibitory effects of the virus on it. Early administration of IFNs could reduce this window of antiviral inactivity and decrease SARS mortality. Combining IFN-g and IFN-α synergistically could maximize this benefit.

Heberon alpha R®

Heberon Alfa R® (IFN alfa-2b) is a drug produced in Cuba by the Center for Genetic Engineering and Biotechnology (CIGB), which has remained a product with proven antiviral efficacy and an adequate safety profile for 34 years[26].

The evidences are different for preventive use and the treatment at early stage of the disease, so Heberon Alpha R is one of the interventions included in the Cuban protocol for the management of COVID-19 and about 76,900 doses have been supplied to the National Health System by the CIGB since March 2020.

The current Cuban recommendation for patients is to use recombinant interferon alfa 2b (Heberon alfa 2b) 3 million of units intramuscular routs thrice a week at early stage.

Today there are several clinical studies with IFN alpha-2b in COVID-19 registered at clinicaltrials.gov and guideline issued by Expert Committees of WHO, Singapore, South Korea and US Institutions recommend the clinical use of IFN alpha for the treatment and prevention of COVID-19. However, the use of IFN in advance stages of the disease has been inconsistent.

HeberFERON®HeberFERON® (IFN-α2b+ IFN-γ) is a drug produced in Cuba by CIGB, which was approved in Cuba by CECMED in 2016 for the treatment of basal cell carcinomas (BCC), and since then it has been routinely administered to more than 3,000 patients with advanced, high-risk or multiple BBC in the country.

HeberFERON® has been extensively studied through physicochemical studies, biological activity in vitro[27] and in vivo[28], in studies of pharmacokinetics, pharmacodynamics in humans[29,30], animals[31]and toxicology,[32].

The proteins, enzymes and metabolites mediating the antiviral effect (2-5 OAS, β-2 microglobulin, Neopterin) of IFNs are synergistically stimulated by HeberFERON[29,30]. The main intracellular signaling factor common to both IFNs, STAT-1[7] has also been synergistically stimulated and has been shown to be a target of SARS-CoV antagonism on the IFN system[12].

These evidences support the use of this therapeutic candidate for the control of COVID-19 in the early stages of the disease and in patients positive for the virus.

We have decided to compare these two formulations of IFNs to define the antiviral of IFN formulations activity against SARS-CoV-2 in a controlled randommized clinical trial.

Objectives {7}

Primary objective:

To assess the efficacy and safety of subcutaneous administration of HeberFERON (combination of IFN-α2b and -γ) and Heberon (IFN-α2b) in patients confirmed to be infected by SARS-CoV-2. Secondary objectives:

- To determine the effect of treatment in terms of the time until SARS-CoV-2 RNA negativization (according to the RT-qPCR technique in real time),
- To determine the proportion of negative results of SARS-CoV-2 post-antiviral therapy,
- To determine the percentage of patients with unfavorable evolution during the execution of the
- To determine serum levels of IFN response markers (2-5OAS, Neopterin, β-2 microglobulin, Mx protein),

- To assess the immune system activation (expression of MHC-I / II, NK cells, cytotoxic T cells, memory cells, and macrophage activation),
- To identify and describe the adverse events that may occur after subcutaneous administration of the antiviral treatment under study

Trial design {8}

The investigation was designed as an open-label, single center, parallel design, 2- arm (allocation ratio 1:1), superiority, randomized controlled trial.

Methods

Participants, interventions and outcomes

Study setting {9}

This is clinical trial in one center. The clinical site is the Central Military Hospital Luis Diaz Soto, in Havana, Cuba.

Eligibility criteria {10}

Eligibility criteria Inclusion criteria

1. Patients to be enrolled must compline the following inclusion criteria:
2. Positive to SARS-CoV-2, symptomatic or not, newly diagnosed as determined by RT-qPCR in oropharyngeal swab
3. ≥ 19 years of age,
4. Functional state according to ECOG ≤ 2 (Karnofsky ≥ 70%). Fully active; no performance restrictions. 1. Strenuous physical activity restricted; fully ambulatory and able to carry out light work. 2. Capable of all self- care but unable to carry out any work activities,
5. Be willing to sign informed consent.

Exclusion criteria

1. Decompensated chronic diseases at the time of inclusion (severe arterial hypertension, ischemic heart disease, diabetes mellitus,);
2. Patients with a history of autoimmune diseases;
3. Presence of hyper-inflammation syndrome,
4. Serious coagulation disorders;
5. Known hypersensitivity to any of the components of the formulation under study;
6. Pregnancy or lactation,

7. Obvious mental inability to issue consent and act accordingly on study.

Who will take informed consent? {26a}

a. Patients will be asked for written consent to participate after having been duly informed about the characteristics of the trial, objectives, benefits and possible risks. They will have the necessary time to decide (minimum 24 hours). Likewise, they will be informed of the right to participate or not and to withdraw their consent at any time, without exposing themselves to limitations for their medical care or other retaliation.
b. The investigator should obtain the patient's written consent only after making sure they understood all the information offered. The procedure will be provided by means of a standard writing; in language easily understood by the patient (it should not be technical, but practical). Neither the investigator nor the trial staff can influence the patient's decision to participate or continue in the trial. All patients who provide informed consent will be randomly allocated into one of two study groups.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

The consent for collection and use of participant data and biological specimens is included in the informed consent

Interventions

Explanation for the choice of comparators {6b}

The CUBAN NATIONAL ACTION PROTOCOL FOR COVID-19[Error! Bookmark not defined.], taking in account the e xperience and results from Chine with the use of Kaletra and chloroquine plus IFN alpha in the treatment of COVID-19 patients, established the obligatory use of this combination for the treatment of SARS-CoV-2 patients. It was not possible to introduce a 3-arm with the sole use of kaletra and chloroquine, because the Cuban health authority prohibited the exclusion of Heberon Alpha R (IFN alpha2b) from the treatment schedule. Then, the combination of Kaletra, chloroquine and IFN alpha 2b was used as comparator.

Intervention description {11a}

Are all medications are administered by hospital staff so the patient has no responsibility for intake Intervention group

The intervention group will receive Kaletra (200/50 mg orally twice daily) and chloroquine (250 mg orally twice daily), as described[Error! Bookmark not defined.], and HeberFERON. HeberFERON will be administered two times per week at 3.5 MIU for 3 weeks. The use of HeberFERON does not contradict the obligatory use of Kaletra and chloroquine, and Heberon Alpha R, because it self-contains IFN alpha2b at the same concentration as used in the comparator.

Control group

The control group will use Kaletra and chloroquine, plus Heberon Alpha R administered three times per week at 3.0 MIU for 3 weeks, as described in the CUBAN NATIONAL ACTION PROTOCOL FOR COVID-19 Version 1.4.**Error! Bookmark not defined.**.

Criteria for discontinuing or modifying allocated interventions {11b}

The allocated intervention may be discontinued by the medical staff or by the patient himself, decision. The patients may withdraw their consent at any time, without exposing themselves to limitations for their medical care or other retaliation.

In the event of serious adverse events (with proven causality) the administration of the product will be interrupted and the required measures will be taken depending on the event and the possible expedited report of the same will be considered. The occurrence of the event will be notified to the monitor within a period not exceeding 24 hours.

The person responsible for the investigation, the monitors and the team of investigators, will carry out the corresponding investigations in order to determine the component causing the undesired effect.

After recovery from the adverse event, the patient may restart treatment according to the treatment scheme has been received. If severe toxicity occurs again, treatment is permanently discontinued.

Strategies to improve adherence to interventions {11c}

Prior to the preparation of this protocol, a criteria unification workshop was held with the participation of clinical specialists and opinion leaders involved in the project, where the experimental evidence in animals and humans, as well as the elements of rationality, were presented and discussed. The analysis, discussion and mastery of the protocol will favor adherence and compliance with GCP by all researchers.

The trial monitors will make quality monitoring visits at all stages of its execution, ensuring strict compliance with the provisions of the protocol.

In the case report form (CRF) there is a table to control the use of the in study drugs that signs the doctor.

Relevant concomitant care permitted or prohibited during the trial {11d}

Rules for the use of concomitant treatments.

Medicines may be used concomitantly to prevent or mitigate adverse events of probable causation with the use of Heberon Alfa R and HeberFERON.

Indications for treatment with antihistamines and / or steroids

Drugs	Habitual doses (adults)
Diphenhydramine	20 mg c/8 h
Hydrocortisone	100 mg c/8 h
Prednisolone	20 mg c/8 h
Dexamethasone	8 mg c/8 h
Chlorpheniramine	4 mg c/8 h
Promethazine	25 mg c/4-6 h

Dipirone or acetaminophen will be indicated to relieve fever, headache, and pain if necessary, depending on the doses listed below, taken from the AHCPR "Guidelines for the Management of Acute Pain"[33].

Indications for treatment with antipyretics / analgesics

Drugs	Habitual doses (adults)
Dipirona	300-600 mg c/4-6 h
Paracetamol	500 mg a 1g c/6-8 h

In the event of nausea or vomiting, gravinol (1 ampoule; 50 mg) or metoclopramide (1 ampoule; 10 mg IM or I.V. every 8 hours) may be administered.

In case of need for other concomitant medication, this will be administered at the discretion of the investigator in charge of the patient. The conduct before any adverse event will be the decision of the responsible investigator and will depend on the type, magnitude and severity of the clinical manifestations in each case.

Provisions for post-trial care {30}

'Not applicable'. There is no anticipated harm and compensation for trial participation. No compensation is provided for enrolment in the trial.

Outcomes {12}

Primary outcomes

Time to 2019-nCoV RNA negativity in patients and the time until progression to severe COVID-19 within 14- 21 days of randomization.

Secondary outcomes.

1. The rate of patients with non-favorable evolution as measured by clinical evaluation (fever, unproductive cough or dyspnea, and their X-ray or CT scan imaging) within 14-21 days of randomization;
2. The increments of RNA and protein levels of IFN responses markers (2-5 OAS, ß-2 microglobulin), and IFN signaling factors (STAT-1 and STAT-3) within 14 days of randomization;

3. Increments in the MHC-I and II and the activation of NK, T cytotoxic and memory cells within 14 days of randomization;
4. Safety within 14-21 days of randomization.

Virological evaluation:

1. Time to negativization of the SARS-Cov-2 RNA (absence of the virus according to the qPCR technique in real time) in positive patients after starting antiviral therapy. The percentage of patients negative to SARS-COV-2 by qPCR in nasopharyngeal exudate tissue will be calculated at 48, 72 and 96 hours after starting

Clinical evaluation:

1. Time to progression to severe COVID-19. The percentage of patients who become severe after the end of the antiviral treatment under investigation (three weeks) will be

Participant timeline {13}

	Study period					
	Base line	**Post allocation**				
Time point (study day)	**DO**	**Week 1**		**Week 2**		**Close-out**
Enrolment and assignment						
Eligibility assessment	X	Day 2	Day 4	Day 9	Day 11	Day 14
Informed consent	X					
Randomized subjects	X					
Baseline data collection	X					
Study drug administration						
Drug dispensing	X ←	—	X	X	X	→
Adverse-drug reaction-assessment						
Serious-adverse-event-assessment						
Clinical-data-collection						
Vital parameters	X	X	X	X	X	X
Body temperature						
Outcomes						X
Chest-X-ray	X	X	X	X	X	X
Electrocardiogram	X ←	—	X	X	X →	X
Laboratory data collection		48h	72h	96h		
SARS-CoV-2 buccal swabs	X	X	X	X		X
Haematological determinations	X	X	X	X	X	
Chemistry	X	X	X		X	

Sample size {14}

The sample size was estimated for the difference in the proportion of negative patients at different time (secondary outcomes), early in the disease, when the effect of external agent can exerts the highest effect, using the PASS software .Taking in account that the 100% of patients will be positive to SARS-

CoV-2 and that only 15% will have the probability to became severe ill, we hope to enroll a sample size of one hundred and twenty patients for this study, based on a power of 80%, and a level of confidence set at 95%, while also considering a dropout rate of 5%.

Recruitment {15}

The source of patients is all the positive patients that are hospitalized at the study hospital positive by RT-qPCR amplification in oropharyngeal swab to SARS-CoV-2.

Assignment of interventions: allocation

Sequence generation {16a}

There will be a centralized randomization at CIGB. The randomization procedure will be carried out by the Supply Group of the Direction of Clinical Investigations at CIGB. Patients will be block randomized individually to one of two treatment arms by means of random computer-generated lists, with an allocation ratio of 1:1, with block sizes of six patients

Concealment mechanism {16b}

The allocation of treatment will be done after the patients have been included following the inclusion and exclusion criteria, and will be mediated by an email with attached allocation form that must include the following information: name and family name initials, clinical history number, age, sex, previous or current concurrent diseases, doctor name, health intuition name and province. It should be send also an electronic copy of writing consent of the patient, signed by the patient and the medial doctor responsible for the study. The allocated vials of the drug will contain etiquette with the inclusion code, the short name of the trial (ESPERANZA), and the lot, and its spired data.

Implementation {16c}

The information of the treatment group (A or B) will only be known after the patient is included. At the time of inclusion (after the selection criteria have been verified and written informed consent has been obtained), the clinical investigator will give the information to the CIGB monitors (who will visit the hospital daily and remain at their posts command). After collecting the general patient information to include (initials, date and time), will assign the inclusion number and the corresponding treatment (consecutive, according to a random list in their possession).

Assignment of interventions: Blinding

Who will be blinded {17a}

This is an open label RCT so patients, doctors are aware of treatment allocation and outcome assessment is not blinded. The virological data analysis will be conducted blind by external laboratory personal and the clinical data analysis by external statistic committee.

Procedure for unblinding if needed {17b}

The design is open label with only data analysts being blinded, so unblinding will not occur

Data collection and management

Plans for assessment and collection of outcomes {18a}

For the conduction of the trial following the Good Clinical Practice, the Steering Committee and any other personal related to the trail execution (laboratory technician, nurses, and pharmacist) will participate in a workshop to receive specific technical information about the products for clinical interventions during the investigation and information and training in procedures for assessment and collection of outcomes, ensuring compliance with the study procedures,

The design, execution of the trial, the clinical and analytical determinations, as well as the analysis of the data will be carried by suitably qualified personnel.

Doctors and nurses were aware of the possible adverse effects of IFNs and their management, both those expected immediately after applying the product (local to the injection site) and hours later (flu-like symptoms).

Routine blood examinations will be performed included whole blood counts, coagulation profiles, serum biochemical tests (including renal and liver function, electrolytes, and coagulation), C-reactive protein (CRP) and ferritin levels.

These determinations will be done using reagents of the required quality and validated techniques. In the case of hematological determinations, several equipment will be used (BC 3200 Hematological Complex, BC 5800 Hematological Complex, as well as a Coagulograph). For blood chemistry determinations, the Midray BS -400 equipment will be used. The reagents to be used for each determination are the following: Gluc, AU, Chol, TRIG, Prot, Fosf, Album, CA, BT, BD, TGO, TGP, FALC, GGT, AML, CPK, CKMB, CREAT, HDLc,

LDLc, Urea, FE, FERRIT, PCR, HS. All the analytical techniques will include the necessary controls to ensure the reliability of the determinations, in accordance with the procedures of each of them.

All the analytical techniques used included the necessary controls to ensure the reliability of the determinations, in accordance with the procedures for each of them..

All patients will be evaluated by a chest X-ray after randomization and before the administration of first dose of the IFNs, and weekly determinations, on days two and four of each week.

Imaging studies will be carried out using the official validate data form. The imaging evaluations are integrated into the XAVIA PACS Medical Image Transmission and Storage System (PACS) for the storage, acquisition, visualization and manipulation of medical images of diagnostic modalities. It is

complemented by the Radiological Information System (RIS) XAVIA RIS, which allows closing the work flow and facilitating the tools for managing the information of patients who are treated.

Throat swab specimens will be transported to a BSL2 certified laboratory at the CIGB for SARS-CoV-2 viral nucleic acid detection by RT-qPCR, using the QIAamp Viral RNA Mini kit (Qiagen,USA), as per the manufacturer's instructions.

Demographic, clinical, laboratory, treatments and outcome characteristics of patients will be extracted from medical record, registered in the case report forms (CRFs) and then entered in duplicate (independently by two operators) for the subsequent process of automatic comparison and cleaning of the database.

The blood determinations data will be kept in the GALEN LAB software, which is an information system for diagnostic means, aimed at managing them, facilitating the request for examinations, recording and evaluation of the results obtained, as well as the generation of statistical information. This software is designed to be used by diagnostic technicians, physicians, and administrative personnel who need to optimize their work and increase their efficiency. It guarantees a strict control of the information, differentiating for each one, the available options according to their responsibility. It is a useful tool, for a possible evaluation by a committee of experts outside the investigation if it is considered necessary

Plans to promote participant retention and complete follow-up {18b}

There is no any patient retention plan due to the complex pandemic situation. Patients that discontinued the treatment will be asked to finish the planned laboratory and clinical evaluations up to day 14 at home. The medical staff from the trial will design a technician and specialist in internal medicine to obtain blood samples for routine haematological and biochemical determinations from the patient as planed in the procedures at home, and will ask for any adverse events.

Data management {19}

For the purposes of this study, a data entry system will be generated at OpenClinica, which is a free software platform for protocol configuration and CRF design, which allows the electronic capture, storage and management of data.

The data entry will be carried out in duplicate (independently by two operators) for the subsequent process of automatic comparison and correction of the bases, necessary for statistical analysis with accurate information from the trial. For debugging errors, the data that does not match the one registered in the original CRF will be corroborated to avoid confusion. The comparison will be repeated until no differences are found between the databases. This process will guarantee the cleanliness of the data included in the databases that will later be used in the statistical analysis. This activity will be recorded, so that it can be traced to national and foreign inspections and / or audits.

Confidentiality {27}

The protocol's medical specialists, the promoter, the monitors and auditors appointed by the promoter will guarantee that the personal data of the subjects included in the protocol are treated in accordance with the provisions established in Law 15/1999 on data protection personal nature and the regulations that develop it. Likewise, the anonymity of the subjects included and the protection of their identity will be maintained; no personal data of the subjects of the protocol will be transferred, except in those circumstances allowed by law.

Monitors and auditors appointed by the promoter may access clinical information and documentation on the subjects included, in order to verify the accuracy and reliability of the data, but must not collect the personal identification data of the subjects. Access to this data should also be provided to the inspectors of the competent health authorities.

The results of the clinical trial, as well as all the work and reports carried out and all the industrial property rights derived from it, are the exclusive property of the promoter. The latter is committed to disseminating them, once the protocol is finished and whether they are negative or positive, in public access media.

The publication of the results, by the medical specialists of the hospital institutions, in scientific magazines or books and the oral presentations or posters at scientific events, workshops or meetings, must be carried out in agreement with the promoting center.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

The promoter center will be in charge of transmitting, operationally, the procedure for the extraction, identification and conservation of the biological samples that will be transferred to the CIGB according to procedures 4.40.121.01 and 4.40.122.01 in force at the Direction of Clinical Research at CIGB.The transfer of samples from the healthcare unit will be the responsibility of the promoter center, which will guarantee the transportation, specialized personnel and resources necessary to carry out these operations with the maximum quality and compliance with GCP, as established by the procedures. in force (4.40.120.01 and 4.40.123.07) and their biosafety protocols.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

The statistical analysis will be done as intention to treat (patients that will receive at least one doses of the in study drugs) and per protocol (patients that will receive at least four doses of HeberFERON or six doses of Heberon Alpha R). We will compare the study endpoints between the two arms using time-to-event methods with the Cox proportional-hazards model. The different categorical variables will be analyzed using the one- way analysis of variance. To describe the efficacy and safety of the IFN regimens, Kaplan-Meier estimates and a multivariate Cox proportional hazards model will be used to

compare severity of patients and adverse events among the two arms during the study period at week four. A p-value of <0.05 will be deemed to confer statistical significance.

Interim analyses {21b}

The promotor did not considered to undertake interim analyses for this RCT because of the expected high rate of inclusion and the necessity to rapid ending of the trial to have results to define clinical decision for the best treatment of patient in the pandemic situation that permit to have a high rate of discharges in the lowest time as possible.

The promotor did not expect any harm that will justifies the anticipated stop of the trial based in the safety of IFNs in this short time and low does intervention schedules.

Methods for additional analyses (e.g. subgroup analyses) {20b}

No additional analyses were planed

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

All the patients that receive at least one doses of the in study interventions will be evaluated by intention treat independent of the non-adherence to the protocol. The missing data will be considered as part of the statistically analysis for demographic data. In the case of virological evaluations, the analyses will be take in account only the evaluated samples, the missing data will be non-considered

In the case of virological determination when two first consecutive samples (24 hour and 72 hours) will be negative by RT-qPCR, the thirst sample (96 hours) will be no evaluated, and the patient will be declared negative.

Plans to give access to the full protocol, participant level-data and statistical code

{31c}

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

Steering Committee

The Steering Committee, led by the principal investigator, will be responsible for overseeing the conduction of the trial, providing training for new sites, ensuring compliance with the study procedures,

addressing challenges that occur at all sites, reviewing serious adverse events and formulating the statistical analysis plan.

For clinical trials at CIGB, the function of coordinating centre is playing by the Direction of Clinical Research that is the responsible for design, endpoint adjudication, data management, statistical analysis and final report for the clinical trial.

Monitors from the Clinical Research Direction at CIGB, Havana, Cuba.

- Iván Campa Legrá. MSc. Monitor
- Yaquelin Duncan Roberts, MD, MSc. Monitor
- Claudia Martínez Lic. Monitor

Data managing.

- Marel Alonso Valdés. Ing. Informatics at Clinical Research Direction

Statically analysis

- José C. Cortiñas Porras. Lic. Mathematics at Clinical Research Direction

Supervisors

- Iraldo Bello Rivero, MSc, PhD. Project Manager at Clinical Research Direction
- Francisco Hernández Bernal. Methodological Assessor at Clinical Research Direction
- Verena Muzio Gonzalez. MD, PhD. Director of Clinical Research Direction
- Laboratory of Genomic and Immunological determinations. Biomedical Research Direction (BRD) at CIGB, Havana,
- Dania M. Vázquez Blomquist, PhD. Genomic evaluations at BRD
- Monica Bequet Romero. PhD. Immunological evaluations at BRD
- Gilda Lemos Perez. MSc. Immunoenzymatic evaluations at BRD
- Gerardo Guillen Nieto. Methodological and Scientific Assessor. Director of Biomedical Research Direction.

Composition of the data monitoring committee, its role and reporting structure {21a}

Monitors from the Clinical Research Direction at CIGB, Havana, Cuba

- Iván Campa Legrá. MSc. Monitor
- Yaquelin Duncan Roberts, MD, MSc. Monitor
- Claudia Martínez Lic. Monitor

The trial monitors will carry out quality monitoring visits at all stages of its execution, ensuring strict compliance with the provisions of the protocol.

The flow of primary protocol information to and from health institution will be guaranteed by the CIGB.

The research product (HeberFERON), CRF and other models will be delivered / collected by the CIGB, directly by the study monitors. The monitors, in the quality monitoring visits, will collect the CRFs of those patients who concluded their participation in the trial. The Supply Group at CIGB is responsible for the adequate supply of medicines and medical supplies, as well as the collection of the product under investigation (dispensed and not dispensed).

The investigator should have a report of the number of patients included, the detected adverse events, the study departures and the causes of these, as well as any other relevant information during the course of the trial, for when they are requested during the quality monitoring visits.

Adverse event reporting and harms {22}

All the information related to the occurrence of adverse events presented in the patients included in the clinical trial will be recorded and described, as explicitly as possible, by the investigators in the Adverse Event Form of the CRF. The type, duration, intensity, severity, causality and the behavior followed will be described, specifying the frequency and dose of the treatment necessary to alleviate them.

The occurrence of the event will be notified to the sponsor monitor within a period not exceeding 24 hours. This must within the first 24 hours of occurrence. The information may be communicated by phone, fax, email or in person. The person responsible for the investigation, the monitors and the team of investigators, will carry out the corresponding investigations in order to determine the component causing the undesired effect.

Notification of adverse events

It will consist of two stages: the immediate notification of all serious adverse events and the reporting of serious and unexpected adverse events in relation to causation, which will be independent of each other.

The Responsible Investigator will notify the CER and the Sponsor will inform CECMED in the following terms.

In the event of a Serious and Unexpected adverse event that causes death or endangers the life of the patient, it will be reported to the CER, CECMED as soon as possible and never after 7 calendar days from the first moment in which the occurrence of the reaction is known.

Immediate notification will be made to the national regulatory agency in Cuba, Center for State Control of the Quality of Medicines (CECMED), within the first 72 hours after the Promoter becomes aware of the serious (serious) and unexpected adverse event and will be mandatory. This model will be filled in duplicated..

Frequency and plans for auditing trial conduct {23}

Project Management Group will meet to review trial conduct weekly during the first month and every 15 day in the second or thirst month. Quality monitoring and control will be carried out by the monitors, quality control managers, consultants and specialists responsible for the trial. These visits will also serve to discuss any aspect of the protocol that the researcher suggests.

Execution check visits will be made daily from the beginning of the study. The first Quality Control visit will be made immediately after the first patient is included.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Protocol amendments during the study will be notified to the sponsor, to the promotor and to the clinical sites, to trail participants and ethic committee. A copy of the revised protocol will be sent to the promotor and to the Investigator Site File. Any deviations from the Protocol will be fully documented using a breach report form. The protocol will be updated in the clinical trial registry.

As responsibility of the clinician's investigators they must to have an archive with the documents coming from the trail execution, as any communication with the promotor, monitor, quality assurance personal, ethic committee, a copy of the protocol and all the amendments to it during the study.

Dissemination plans {31a}

The final results of the study will be presented in a final report workshop with the participation of all the clinician's investigators and other technical personal involved in the trial executions and supervisors, as well as monitor and other specialist involved from the promotor. The personal eel involved direct achievements of the results (clinical, virological, clinical laboratory, and others) will have the right to communicate the respective results in conferences, national and international meeting, always with the consent of the promotor

Discussion

For many emerging infectious diseases patients are often treated with therapeutics minimal evidences. Due to the urgency of COVID-19 pandemic, not only IFN alpha, but various drugs are being used as therapeutic tools, even though their efficacy has not been completely demonstrated for the treatment of SARS-CoV-2. If these patients had been included in a properly designed study, conclusive evidence might have been generated. Performing such a trial meets a critical need.

Some limitations to our study design should be noted. The blinding was not feasible, it will maintained for laboratory SARS-CoV-2 RNA detection by PCR, that is one of the endpoint of the study.

Trial Status

The HOPE trial has already been approved by the Institutional Review Boards at “Luis Diaz Soto” Hospital and Regulatory Authority for Medicines, CECMED. Enrollment for this study began in April 11, 2020, and has enrolled one hundred patients as of May 26, 2020. The recruitment has been completed in the second week of June 2020.

Abbreviations

AE	Adverse Event
BCC	Basal Cell Carcinoma
CECMED	Center for State Control of the Quality of Medicines
CIGB	Center for Genetic Engineering and Biotechnology
CRF	Case Report Form
CSF	cerebral spinal fluid
CTCEA	Common Terminology Criteria for Adverse Events
ECOG	Eastern Cooperative Oncology Group
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IND	investigational new drug application
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
MERS	Middle East Respiratory Syndrome
MIU	Million international Units
PD	disease progression
PK	Pharmacokinetics
SARS	Severe Acute Respiratory Syndrome

Declarations

Acknowledgements

We thank Drs. Esperanza Amoroto (National Centre for the Coordination of Clinical Trials) and Jorge de Jesús Menéndez Hernández (CECMED), for all the recommendations to amend the protocol. This work was supported by Center for Genetic Engineering and Biotechnology and Ministry of Health of Cuba.

Authors' contributions {31b},

IBR, conception and design, analytical plan, drafting of the manuscript, critical revision of the manuscript for important intellectual content, approval of the final version to be published and agreement to be accountable for all aspects of the work. FHB, critical revision of the manuscript for important methodologically content, approval of the final version to be published. HNC, drafting of the manuscript. YDR, analytical plan, approval of the final version to be published and agreement to be accountable for all aspects of the work. CMS, analytical plan. ICL, critical revision of the manuscript for important methodologically content. IEM, clinical research coordinator at the hospital, analytical plan. VMG, approval of the final version of the protocol. GNG, revision of the manuscript for important intellectual content.

Funding {4}

This work was supported by Center for Genetic Engineering and Biotechnology and Ministry of Health of Cuba.The funder played no part in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication

Availability of data and material {29}

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate {24}

The HOPE study is approved by the Ethics Committee of "Luis Diaz Soto" Hospital in Havana, Cuba and by CECMED

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Council for Harmonization–Good Clinical Practice guidelines. Informed consent will be obtained from patients. Site investigators will explain the objectives of the trial and its potential risks and benefits to patients during the process of obtaining consent. No compensation is provided for enrollment in the trial. Patient personal data are de-identified.

Consent for publication {32},

The consent for publication is included in informed consent that sings the patient

Declaration of Informed Consent for the subject

I (name and surname)

....

I have read the information sheet given to me.

I have been able to ask questions about the study.

I have received enough information about the study.

I have spoken with:

....

(name of researcher)

I understand that my participation is voluntary.

I understand that I may withdraw from the study:

1st Anytime

2º Without having to give explanations.

3º Without this having an impact on my medical care.

I freely give my consent to participate in the study and **give my consent for the access and use of my data under the conditions detailed in the information sheet.**

- I agree that the blood or tissue samples obtained for the study may be used in the future for new analyzes related to the disease or study drugs not provided for in the current protocol (genetic analyzes are excluded, as long as they are not part of it of the study objectives):

Competing interests {28}.

Authors IBR, FHB, HNC, YDR, CMS, ICL, VMG and GGN, are employees of the Center for Genetic Engineering and Biotechnology, Havana network where Heberon Alpha R and HeberFERON are produced. The other authors have no competing interests.

References

1. Coronavirus 2019-nCoV Infection: Expert consensus on guidance and prevention strategies for hospital pharmacists and the pharmacy workforce (1st Edition) Coronavirus 2019-nCoV Infection. https://www.scribd.com/document/454346862/Coronavirus-guidance-update.
2. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). Chinese Medical Journal 2020; 133: 1087-1095. doi: 1097/CM9.0000000000000819.
3. Guides for diagnosis and treatment protocol for novel Corona virus pneumonia from John Hopkins Medical Center, www.hopkinsguides.com
4. Manejo clínico del COVID-19 - Ministerio de Sanidad.www.mscbs.gob.es

5. Report of the WHO-China join mission on Coronavirus disease 2019 (COVID-19). https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf.
6. Ozato K, Tailor P, and Kubota, TJ. The Interferon Regulatory Factor Family in Host Defense: Mechanism of Action. Biol. Chem. 282 (28) 20065-20069; Published, JBC Papers in Press, May 14, 2007, DOI 10.1074/jbc.R700003200
7. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev 1998;67:227-64.
8. Tang F, Liu W, Zhang F, Xin ZT, Wei MT, Zhang PH, et al. IL-12 RB1 genetic variants contribute to human susceptibility to severe acute respiratory syndrome infection among Chinese. PLoS One. 2008;3(5):e2183.
9. Mon A, Salmeron OJ, Manzano L, y Rodríguez-Zapata, M, Reyes E, Moltó S. Vaquer L et al. Alpha Interferon as an immunomodulator in the treatment of patients with tumors. Med Oncol. 1995; 12(1):15-21.
10. Tyring S.K. Antitumor actions of interferons: Direct, indirect and synergy with other treatment modalities. Int J. Dermatol. 1987; 26: 549-56.
11. Muller U, Steinhoff U, Reis LF, et al. Functional role of type I and type II interferons in antiviral defense. Science. 1994;264:1918–1921.
12. Frieman M, Yount B, Heise M, Kopecky-Bromberg SA, Palese P, Baric RS. Severe Acute Respiratory Syndrome Coronavirus ORF6 Antagonizes STAT1 Function by Sequestering Nuclear Import Factors on the Rough Endoplasmic Reticulum/Golgi Membrane. J Virol. 2007;81(18):9812–24.
13. Strayer D, Dickey R, Carter W. Sensitivity of SARS/MERS CoV to Interferons and Other Drugs Based on Achievable Serum Concentrations in Humans. Infect Disord – Drug Targets. 2014; 14(1):37–43.
14. Daczkowski CM, Dzimianski J V, Clasman JR, Goodwin O, Mesecar AD, Pegan SD. Structural Insights into the Interaction of Coronavirus Papain-Like Proteases and Interferon-Stimulated Gene Product 15 from Different Species. J Mol Biol. 2017;429(11):1661–
15. Frieman M, Yount B, Heise M, Kopecky-Bromberg SA, Palese P, Baric RS. Severe Acute Respiratory Syndrome Coronavirus ORF6 Antagonizes STAT1 Function by Sequestering Nuclear Import Factors on the Rough Endoplasmic Reticulum/Golgi Membrane. J Virol. 2007;81(18):9812–24.
16. Devaraj SG, Wang N, Chen Z, Chen Z, Tseng M, Barretto N, et al. Regulation of IRF-3- dependent innate immunity by the papain-like protease domain of the severe acute respiratory syndrome coronavirus. J Biol Chem. 2007; 282:32208–
17. Kamitani W, Narayanan K, Huang C, Lokugamage K, Ikegami T, Ito N, et al. Severe acute respiratory syndrome coronavirus nsp1 protein suppresses host gene expression by promoting host mRNA degradation. Proc Natl Acad Sci USA. 2006; 103, 12885–12890.
18. Kopecky-Bromberg SA, Martinez-Sobrido L, Frieman M, Baric RA, Palese P. Severe Acute Respiratory Syndrome Coronavirus Open Reading Frame (ORF) 3b, ORF 6, and Nucleocapsid Proteins Function as Interferon Antagonists. J Virol. 2007; 81(2): 548–557.

19. Chen X, Yang X, Zheng Y, Yang Y, Xing Y, Chen Z. SARS coronavirus papain-like protease inhibits the type I interferon signaling pathway through interaction with the STING-TRAF3-TBK1 complex. Protein Cell. 2014; 5(5):369–381.
20. Channappanavar R, Fehr AR, Zheng J, Wohlford-Lenane C, Abrahante JE, Mack M, et IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. J Clin Invest. 2019;129(9):3625–39.
21. . Hooks J.J, Yun Wang Y, Detrick The Critical Role of IFN-gamma in Experimental Coronavirus Retinopathy. Invest Ophthalmol Vis Sci. 2003; 44:3402–3408.
22. Sainz B, Mossel EC, Peters CJ, Garry RF. Interferon-beta and interferon-gamma synergistically inhibit the replication of severe acute respiratory syndrome-associated coronavirus (SARS-CoV). Virology. 2004; 329: 11–17.
23. Mossel EC, Sainz B, Garry RF, Peters CJ. Synergistic inhibition of SARS-coronavirus replication by type I and type II IFN. Adv Exp Med Biol. 2006;581:503–
24. Larkin J, Jin L, Farmen M, Venable D, Huang Y, Tan SL, et al. Synergistic antiviral activity of human interferon combinations in the hepatitis C virus replicon system. J InterfCytokine Res. 2003;23(5):247–
25. Zhang XN, Liu JX, Hu YW, Chen H, Yuan ZH. Hyper-activated IRF-1 and STAT1 contribute to enhanced interferon stimulated gene (ISG) expression by interferon alpha and gamma co-treatment in human hepatoma cells. Biochim Biophys Acta. 2006;1759(8– 9):417– DOI: 10.1016/j.bbaexp.2006.08.003.
26. Nodarse-Cuní H, López-Saura P. Cuban interferon alpha-2b. Thirty years as an effective and safe drug. Biotecnol Apl, ene.-mar. 2017,34:1211-1217.medigraphic.com.
27. Bello C, Dania Vázquez-Blomquist D, Miranda J, García Y, Inés-Novoa L, l Palenzuela D, et al. Regulation by IFN-α/IFN- Co-Formulation (HerberPAG®) of Gene Involved in Interferon-STAT-Pathways and Apoptosis in U87MG. Current Topics in Medicinal Chemistry. 2014; 14:351-358.
28. Bello I, Garcia Y, Duncan Y, Vazquez D, Santana H, Besada V, et al. HeberFERON, a new formulation of IFNs with improved pharmacodynamics: Perspective for cancer treatment. Seminars in Oncology. 2018; 45: 27–33.
29. García-Vega Y, García-García I, Collazo-Caballero SE, et al. Pharmacokinetic and pharmacodynamic characterization of a new formulation containing synergistic proportions of interferons alpha-2b and gamma (HeberPAG) in pa- tients with mycosis fungoides: an open-label trial. BMC Pharmacol Toxicol. 2012;13:20.
30. García-García I, Hernández-González I, Díaz-Machado A , et al. Pharmacokinetic and pharmacodynamic characterization of a novel formulation containing co–formulated interferons alpha-2b and gamma in healthy male volunteers. BMC Pharmacol Toxicol. 2016;17:58 .
31. Castro J, Puente P, Hernández A, Martínez R, Aldana L, Bello I, et al. Effects of two formulations containing the IFN α2b and IFN on the main clinical, hematological and biochemical parameters in non- human primates from Chlorocebus aethiops sabaeus species. Biotecnología Aplicada.

2015;32:4231-4236. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1027-28522015000400004&lng=es.

32. Cabal C, González E, Bacardí, D, Bello I, Romero JC, Madrigal R, et al. MRI rat organ Assessment under recurrent Interferon The IJES.2016; 5:45-52. https://www.researchgate.net/publication/302590563_MRI_rat_organ
33. AHCPR. Guías para el manejo del dolor agudo. Publicación No. 92-0032. Disponible. www.unisanitas.edu.co/Revista/66/03Rev_Medica_Sanitas_21-1