Projeto de Estudo Clínico de AZVUDINE

Title of the research project	**EVALUATION OF THE SAFETY AND CLINICAL EFFICACY OF AZVUDINE: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY IN MILD-STAGE SARS-COV-2 VIRUS-INFECTED PATIENTS.**
Internal protocol number	**IGZ-2**
Sponsor	HRH Pharmaceutical Limited represented by GALZU INSTITUTE of research, teaching, applied science and technology (ORPC - Representative Organization for Clinical Research).
Proponent Institution	Clinical Research Unit / High Complexity Center (UPC/CAC) / Clinical Research Department / Galzu Institute
Principal Investigator	Carlos Augusto de Araújo Tavares

Co-participating units	**Sub-Investigators***
1. **Santa Casa de Misericórdia de Campos dos Goytacazes-RJ**	Cleber Gloria Silva
2. **São José Pre-Hospital Unit Campos dos Goytacazes-RJ**	Bruno Hanry Fonseca Ribeiro de Lima Viana João Manoel Paravidino de Souza
3. **Armando Vidal Hospital, São Fidelis-RJ**	Antonio Rodrigo Serra Santarém
4. **Moacyr Gomes de Azevedo Hospital, Cambuci-RJ**	Tiago Carvalho Velasco
5. **Hospital de Itaocara-RJ**	Elenio Melo de Almeida

*Physicians responsible for conducting the study in each health unit, under the coordination of the Researcher in charge.

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CONFIDENTIALITY AND COMPLIANCE

This protocol was prepared and will be conducted in accordance with the standards established by the ICH Guidelines for Good Clinical Practice GCP/ICH - ICH Topic E6 (R2) (2016), Americas Document (2005), Resolution 466/12 of the National Health Council and related, RDC 09/15 of ANVISA and related and other applicable regulatory requirements. The data obtained will be used only for purposes determined in this research, keeping the confidentiality of data and results obtained ensured by controlled access of persons responsible for its evaluation and execution.

This document contains trade secret information that is privileged or confidential and should not be disclosed unless such disclosure is required by official or regulatory bodies. This protocol may not be modified or used without the consent of **GALZU INSTITUTE of research, teaching, science and applied technology.**

Sponsor	**HRH Pharmaceutical Limited represented by GALZU INSTITUTE of research, teaching, applied science and technology (ORPC - Representative Organization for Clinical Research).**

HISTÓRICO DE REVISÃO

Versão	Descrição	Data
0.1	Version approved by the Investigator and Sponsor	12/03/2021
0.2	Version approved by the Investigator and Sponsor	05/05/2021
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0.4	Version approved by the Investigator and Sponsor	25/08/2021
0.5	Version approved by the Investigator and Sponsor	03/09/2021
0.6	Version approved by the Investigator and Sponsor	20/10/2021

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SUBSCRIPTION PAGE

The signatures of the Principal Investigator and the Sponsor below constitute agreement on this protocol and provide the necessary guarantees that this study will be conducted in accordance with all specifications described in the applicable laws and regulations and with the ethical principles described in the Declaration of Helsinki, Americas Document (2005), GCP/ICH Good Clinical Practice - ICH Topic E6 (R2) (2016), and in compliance with Resolutions No. 466/12 of the National Health Council - Ministry of Health and related, RDC No. 09/15 of ANVISA and related, as well as other applicable regulatory requirements.

The Principal Investigator and Sponsor are aware that implementation of the study protocol and documents can only be done after all necessary ethical and regulatory approvals have been obtained and the Sponsor has authorized the initiation of the study.

Rio de Janeiro, October 20, 2021

Dr. Carlos Augusto de Araújo Tavares Clinical Research Unit / CAC Principal investigator CPF: 470.611.037-87	Prof. Dr. Paula Gelas Abreu Cabral Galzu Institute / ORPC of HRH Pharmaceutical Limited Presidente / CPF: 859888127-91

LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
Alb	Albumin
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase (TGP)
AST	Aspartate Aminotransferase (TGO)
BASO #	Number of Basophils
BI	Researcher's brochure
BNP	B-type natriuretic peptide
BPC	Good Clinical Practice
CHE	Cholinesterases
CIMS	Independent Data Monitoring and Security Committee
CK	Creatine kinase
Cl	Chlorine
COVID-19	Corona Virus Disease 2019
CRP	C-Reactive Protein
DBIL	Direct Bilirubin
DM	Data Manager
DMP	Data Management Plan
DNA	Deoxyribonucleic Acid
EA	Adverse Event

EAG	Serious Adverse Event
ECA2	Angiotensin-converting enzyme 2
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EFV	Efavirenz
EOS #	Number of Eosinophils
ESR	Red cell sedimentation
FAS	Full Analysis Set
FDA	*Food and Drug Administration*
FIB	Fribrinogen
FR	Respiratory Rate
GGT	Gamma-Glutamyl Transferase
Hb	Hemoglobin
HCT	Hematocrit
HIV	Acquired Immunodeficiency Syndrome
ICH	*International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use*
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukin-6
IWRS	*Interactive Web Response System*

K	Potassium
LYMPH#	Number of Lymphocytes
MCH	*Mean Corpuscular Hemoglobin*
MCHC	*Mean Corpuscular Hemoglobin Concentration*
MCV	*Mean Corpuscular Volume*
MONO #	Number of Monocytes
Na	Sodium
NEUT#	Number of Neutrophils
NMPA	*National Drug Administration*
NOAEL	*no Observed Adverse Effect Level*
OMS	World Health Organization
PAM	Mean blood pressure
PaO2	Arterial oxygen pressure
PAS	Systolic blood pressure
PCT	Procalcitonin
PI	*Principal Investigator*
PLT	Platelet count
POP	Standard Operating Procedure
PPS	*Per Protocol Set*
RBC	*Red blood cell count*
RNA	Ribonucleic acid
SatO2	Oxygen Saturation

SCR	Serum Creatinine
SS	*Safety Data Set*
SUSAR	*Suspected Unexpected Serious Adverse Reaction*
TBIL	Total bilirubin
TC	Computed tomography
TCLE	Free and Informed Consent Term
TDF	Tenofovir
TMPRSS2	Transmembrane serine protease 2
TP	Total proteins
TP	Prothrombin time
TT	Thrombin time
TTPA	Activated Partial Thromboplastin Time
UA	Uric Acid
UREA	Urea
VM	Mechanical Ventilation
WBC	*White Blood cell count*

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1. SUMMARY

SYNOPSIS

Title	Evaluation of the safety and clinical efficacy of AZVUDINE: Randomized, double-blind, placebo-controlled study in **mild-stage patients** infected with the SARS-CoV-2 virus.
STUDY ID	IGZ-2
DOCUMENT TYPE	Phase III Clinical Trial Design
VERSION/ISSUE DATE	Version 0.5 of September 3, 2021
SPONSOR	HRH Pharmaceuticals Limited, HONG KONG / 3037435, 1201, 12/F TAI SANG BANK, BUILDING 130-132 DES VOEUX ROAD, CENTRAL, HONG KONG.
REPRESENTATIVE ORGANIZATION OF CLINICAL RESEARCH SPONSOR IN BRAZIL (ORPC)	GALZU INSTITUTE of Research, Teaching, Science and Applied Technology, CNPJ: 28.831.817/0001-20. Lourival Martins Beda, 1082 - Donana, Zip Code: 28110-000, Campos dos Goytacazes-RJ. Pelinca, 115 - Parque Tamandaré, 3º andar, CEP: 28035-053, Hospital Santa Casa de Misericórdia de Campos, Phone: (22) 2726 6550 extension 180/194 Contact Paula Cabral (22) 98126 6602 Site: http://www.institutogalzu.org.br E-mail: institutogalzu@galzu.org.br
PROPOSING INSTITUTION	Clinical Research Unit / High Complexity Center (UPC/CAC) / Clinical Research Department / Galzu Institute
PRINCIPAL INVESTIGATOR	Dr. Carlos Augusto de Araújo Tavares

CO-PARTICIPATING INSTITUTIONS	1. Santa Casa de Misericórdia de Campos, Campos dos Goytacazes-RJ 2. Unidade Pré Hospitalar São José, Campos dos Goytacazes-RJ 3. Hospital Armando Vidal, São Fidelis-RJ 4. Hospital Moacyr Gomes de Azevedo, Cambuci-RJ 5. Hospital de Itaocara, Itaocara-RJ
OBJECTIVE	**PRIMARY OBJECTIVE:** • To evaluate the **efficacy** and **safety of** AZVUDINE (FNC) in patients infected with mild stage SARS-COV-2; [Period: 14 days]. **SECONDARY OBJECTIVE:** • To evaluate the **clinical outcome of** participants infected with mild-stage SARS-CoV-2 treated with AZVUDINE (FNC) versus placebo.
INTERVENTION	• Experimental group: AZVUDINE 5mg/day, orally for up to 14 days + Standard treatment. • Control group: PLACEBO orally for up to 14 days + Standard treatment.
NUMBER OF PARTICIPANTS	312 participants randomly and equally divided in the two arms of the study (156 practitioners in each group), considering a 20% dropout.
TRIAL DESIGN	The study consists of 3 periods: **screening, treatment and follow-up**. In the screening period, patients who meet the eligibility criteria will be included in the study and randomized into one of the two study arms,

	experimentalor control. The first day of treatment will be day 1, and will last up to 14 days. **Treatment will be terminated early when two consecutive RT-PCR tests are negative (one of the criteria for outpatient discharge).** Treatment will also be stopped early in the event of participant drop-out or in the event of an EAG. Safety follow-up will be performed at 28 and 60 days after outpatient discharge. For **Early Termination (ETA)** of treatment, participants should follow the routine for visit 17: End of Treatment (D15), in advance. The study will be considered completed when the last participant completes their last visit.
INCLUSION CRITERIA	Individuals who present the following characteristics will be included in this study: 1. Age ≥18 years, regardless of gender; 2. Fluorescence RT-PCR test result of respiratory or blood samples must be positive for COVID-19, or viral gene sequencing of respiratory tract samples must be highly homologous to COVID-19; Individuals with COVID-19 must meet the diagnostic criteria in the "latest version of clinical guidelines for COVID-19" issued by the World Health Organization (WHO) on June 4, 2020; 3. Symptomatic patients who meet the case definition for COVID-19, according to WHO, without evidence of

	bacterial pneumonia or hypoxia (Sat O2 < 95%)[1] [score 1-3]; 4. Voluntary participation and signing of the informed consent form.

[1] Signs and symptoms presenting with COVID-19: Most people present with fever (83-99%), cough (59-82%), fatigue (44-70%), anorexia (40-84%), shortness of breath (31-40%), myalgias (11-35%). Nonspecific symptoms: sore throat, nasal congestion, headache, diarrhea, nausea, vomiting, loss of smell (anosmia) and loss of taste (ageusia). Neurological manifestations include: dizziness, agitation, weakness, seizures, problems with speech or vision, sensory loss, or problems with balance in standing or walking. WHO, Clinical management of COVID-19: living guidance, 25 January 2021, Table 6.1, p.19.

EXCLUSION CRITERIA	Individuals who present one or more of the following characteristics will not be eligible to participate in this study: 1. Know or suspect that you are allergic to any of the components of AZVUDINE tablets (inactive ingredients: microcrystalline cellulose, lactose hydrate, polyvinylpyrrolidone K30, croscarmellose sodium, magnesium stearate); 2. Individual presenting shortness of breath and Sat O2 < 95%; or any other symptom requiring treatment through hospital admission; 3. Patients with liver disease (total bilirubin ≥2mg/dL, ALT/TGP e AST/TGO ≥5 times above normal limit);[2] 4. Pactients with a history of known liver disease (cirrhosis with ChildPugh classification B and C); 5. Patients with a history of renal insufficiency (glomerular filtration rate ≤60mL/min/1,73m^2); 6. Patients with history of congestive heart failure (NYHA ¾ grade), untreated symptomatic arrhythmias ormyocardial infarction within 6 months; 7. Individuals with malabsorption syndrome, or other conditions affecting gastrointestinal absorption, and circumstances in which patients require intravenous nutrition, or cannot take medications orally or nasogastrically; 8. Total neutrophil count <750 cells/L; 9. Women who are pregnant or lactating, or of childbearing potential during the study period and within 6 months after termination of administration; 10. Subjects who have participated in another clinical trial and used any experimental drug within the last 12 weeks;

[2] Normal values: BT up to 1.2 mg/dL; TGO Adult 12 to 46 U/L; TGP Adult 03 to 50 U/L; GGT Man 10 to 50 U/L, Woman 07 to 32 U/L (Source: Instituto Hermes Pardini).

CRITERIA FOR DISCONTINUATION OF THE STUDY	The following criteria define **study discontinuation for the participant:** 1) Voluntary withdrawal by the participant at any time and without any prejudice to his/her health care; 2) Any situation, at the discretion of the Principal Investigator, that puts the safety of the participant at risk; 3) Refusal or inability to perform and/or conclude imaging and laboratory tests, when applicable; 4) Loss of contact with the participant for follow-up; 5) Death; 6) Outpatient discharge (2 negative RT-PCR tests). The following criteria define the **discontinuation of the study** as a whole: 1) Termination of the trial by the sponsor, provided that this is justified; 2) Termination of the study by the Principal Investigator, provided that this is justified; 3) Termination of the study at the request of local ethical and health authorities.
PROCEDURES	Demographic data collection, physical examination, vital signs assessment, blood collection, respiratory tract sampling, imaging, ECG and pregnancy test (female participants) will be performed.
OUTCOMES/ENDPOINTS	**PRIMARY ENDPOINT:**

	• Proportion of patients hospitalized during the study up to day 28 according to WHO ordinal scale of clinical progression (Jun/2020), Score 4 to 10.[Period: until D15, D21 and D28, approximately 14- 30 days]. **SECONDARY OUTCOME:** • Proportion of participants with clinical outcome of **CURE** during the study; [Period: until D15, D21 and D28, approximately 14-30 days]. ○ The clinical outcome of cure is defined in this protocol as the absence of viral RNA in collected samples and clinical conditions for outpatient discharge[3]. • Improvement in clinical status in at least one category compared to screening on the Ordinal Scale of Clinical Improvement (WHO, Jun/2020) [Period: until D15, approximately 14 days]. • Severity and duration of symptoms: fever, cough, fatigue or tiredness, shortness of breath, myalgia, nasal congestion or runny nose, sore throat, headache, chills, nausea, vomiting, anosmia, ageusia[4]; [Period: until D15, D21 and D28, approximately 14-30

[3] According to the WHO Working Group on Characterization and Clinical Management of COVID-19 Infection (July, 2020), clinical and virological absence of infection (non-detectable RT-PCR) is suggestive of CURE for initially infected participants.

4 Assessing COVID-19-Related Symptoms in Outpatient Adult and Adolescent Subjects in Clinical Trials of Drugs and Biological Products for COVID-19 Prevention or Treatment Guidance for Industry, FDA September 2020, https://www.fda.gov/media/142143/download

	days] • Changes in liver and renal function baseline; [Period: until D15, D21 and D28, approximately 14-30 days] ○ Laboratory tests performed on days D1, D3, D5, D7, D9, D13, D15, D21, D28 and D60 • Time of AZVUDINE use until the second negative RT-PCR conversion [Period: until D15, approximately 14 days]. • Evaluation of negative conversion time of SARS-CoV-2 viral load by RT-PCR between AZVUDINE (FNC) group and control group; [Period: until D15, D21 and D28, approximately 14-30 days]. • Evaluation of the number of cycles for the detection of viral load of SARS-CoV-2 by RT-PCR and application of the standard curve for calculation of viral load; [Period: until D15, D21 and D28, approximately 14-30 days]. • Analysis of the relationship between the calculated and/or quantified viral load and the clinical evolution of the participants in the AZVUDINE (FNC) group compared to the control group; [Period: until D15, D21 and D28, approximately 14-30 days]. • Occurrence of drug interactions; [Period: until D15, D21 and D28, approximately 14-30 days]. • All-cause mortality rate during the study; [Period: until D15, D21, D28 and D60, approximately 14-60 days]. • Frequency and intensity of adverse events, unexpected adverse events, and serious adverse

	events; [Period: until D15, D21, and D28, approximately 14-30 days]
	• Proportion of comorbidity-related worsening [Obesity, diabetes, alcoholism, smoking, lung disease]; [Period: until D15, approximately 14 days].
	• Aggravations occurring in the post-treatment period, up to 60 days or outcome. Period: until D60 [approximately 60 days]
	• To evaluate the tolerability of AZVUDINE (FNC) use at 5mg/day regimen for up to 14 days: [Period: until D15, approximately 14 days].
	  ○ Calculating treatment adherence;
	  ○ Total time of use of AZVUDINE (FNC);
	  ○ Proportion of participants who withdrew consent;
	  ○ Proportion of participants who abandoned treatment.

ANALYSES	All statistical tests are performed by two-sided test. A significance level of 5% shall be adopted. Detailed statistical methods will be provided in chapter 11.
LENGTH OF STUDY	From the approval of the study by the Research Ethics Committee and ANVISA, it is estimated the period of approximately 6 months for its completion.

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FLUXOGRAM

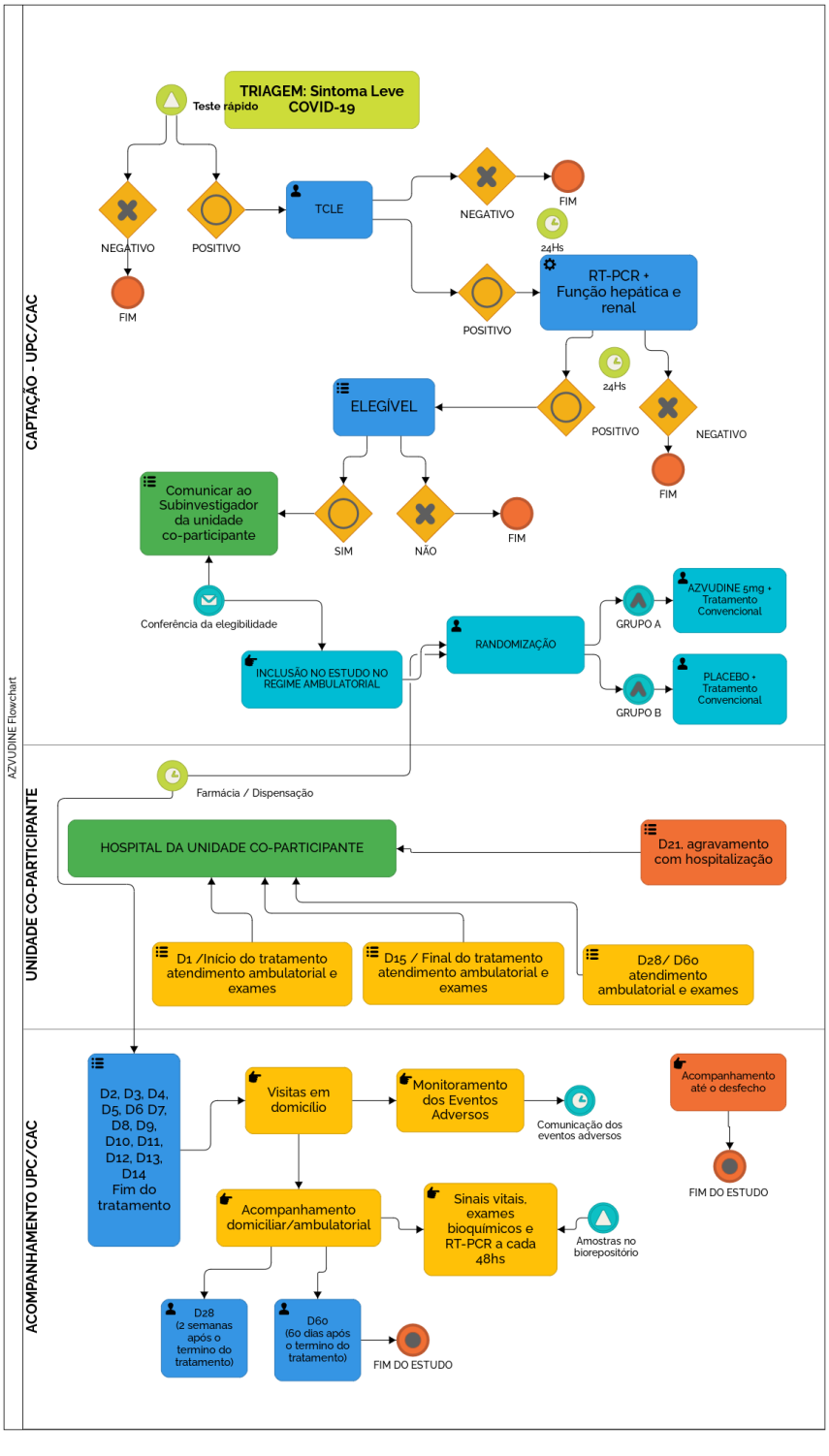

Figure1: General flowchart of the study

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STUDY SCHEDULE

The research project will follow the execution schedule, which comprises a total period of 6 months, as of the document confirming registration of the research at Plataforma Brasil and release for execution by the CEP/CONEP system. Taking into account the deadlines established for ethical analysis by the CEP/CONEP system, as set forth in the Operational Rule 001/2013 of the National Health Council and ANVISA Resolutions, the following schedule is proposed:

	Steps	Description	Plannedperiod
ELABORATION	Preparationofthe project and othernecessary documents	Preparationoftheresearchproject,brochure,TCLE,ethicalandregulatorydossier	March 2021
	Regulatory Approvals	Submissionofthedossier forethicalevaluationby theCEP/CONEPSystem	April to June 2021
		DrugDevelopmentPlanupdatetoDDCMANVISA: 25351166460202183	March 2021
	ThebeginningoftheactivitiesoftheResearchProjectwillonlyoccuraftertheapprovaloftheIRB, reasonwhyitwasnotpossibletoestimateaperiod,butthetimerequiredfortheconductionofeachstep		
CONDUCTION	Recruitment	Outreachandrecruitmentofresearchparticipants	October2021
	Screening	Invitationtotheparticipant ApplicationandsigningoftheTCLEEligibility Criteria	October 2021 to January 2022
	Datatreatmentandcollection	Treatmentofresearchparticipantswithan investigationaldrugorPLACEBO,concomitantwithstandardtreatment forCOVID-19	October 2021 to January 2022
CONCLUSION	Evaluationof results andstatisticalanalysis	Correlationsbetweentheparametersmeasuredandtheclinical data	February 2022
	Finalstudyreport	Contextualizationanddiscussionoftheresearch findings	February to March2022
	Publicationof results	Disseminationofresultstothescientific community	April 2022

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It is noteworthy that the dates may be changed depending on the deadlines for ethical and regulatory review. In case of approval before the schedule, the inclusion of participants and data collection will be anticipated.

2. INTRODUCTION

COVID-19

In December 2019, the world observed the emergence of unexplained cases of pneumonia in Wuhan, China. These cases were associated with a new virus of the coronaviridae family, identified in January as SARS-CoV-2, which causes the disease COVID-19 (Corona Virus Disease 2019) (VELAVAN; MEYER, 2020). According to the World Health Organization (WHO), transmission can occur through contact, breath or droplets generated during coughing, sneezing or enunciation, which can remain in the air for long distances and time, particularly in poorly ventilated environment, and somehow come into contact with mucous membranes (WHO, 2020). The mean incubation time of SARS- CoV-2 is around 5 days, ranging from 2 to 14 days(WHO, 2020d). The most common symptoms are fever, cough, runny nose, sore throat, shortness of breath, loss of smell, taste alteration, fatigue and decreased appetite (WHO, 2020). The clinical picture of infected people can vary from an asymptomatic state or common cold to a severe state of pneumonia, which can lead to respiratory failure and death (SINGHAL, 2020). According to the World Health Organization, the mild state is characterized by symptoms such as fever, cough, loss of smell, among others, but without evidence of pneumonia or hypoxia. The moderate state is characterized by non-severe pneumonia and the severe state is characterized by severe pneumonia and severe respiratory difficulty. Patients may evolve to a critical stage of the disease, which is characterized by worsening of respiratory symptoms, sepsis or septic shock, and may lead to death(WHO, 2020a).

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"Until June 2, 2021, the Ministry of Health registered 16,720,081 confirmed cases and 467,706 deaths. The state of Rio de Janeiro registered 874,560 confirmed cases and 51,096 deaths(Epidemiological situation of COVID-19 in Brazil - Ministry of Health). The risk map of Covid-19 (05/28/2021) shows that the state of Rio de Janeiro, including Norte Fluminense, is flagged orange for the contagion of the disease. In Campos dos Goytacazes - RJ, the largest city in the interior of Rio de Janeiro, this yellow flag continues to receive patients from the macroregion, besides the lakes region and Baixada Fluminense, which contributes to the lack ofhospitalization vacancies in the SUS network. The occupancy of ICU beds in the SUS and private network reached 82.88% and the occupancy rate of Internal Medicine beds in the SUS and private network is 68.08% on June 1st, 2021. Besides, we have the Indian strain as a third wave concern. Our city concentrates the medical care of the entire region by housing regional/state hospitals and reference in several specialties and in addition, the Brazilian scenario does not tend to stabilize.

Coronaviruses have three surface proteins: envelope protein, membrane protein and the S-glycoprotein (Spike), the latter contributing to host receptor binding, cell tropism and pathogenesis. The S proteins form a triomer generating a crown-shaped structure, which explains the name of this virus family (corona = crown) and are functionally divided into the S1 and S2 domains. In the process of entry into human cells, SARS-CoV-2 binds to angiotensin converting enzyme type 2 (ACE2) through the S1 domain with the help of Transmembrane Serine Protease-2 (TMPRSS2) and the membrane fusion is performed by the S2 domain. Subsequently, the viral genomic RNA is released into the cytoplasm of infected cells. One of the first steps of coronavirus processing is the cleavage of pp1a and pp1ab polypeptides by a protease, forming a protein complex responsible for viral RNA replication and transcription. The pp1a protein complex is formed by viral RNA polymerase (RdRp), helicase and other functional enzymes related to viral RNA modification (Romano et al., 2020). After replication and subgenomic RNA synthesis, viral structural proteins are translated and inserted into the endoplasmic reticulum. The viral genomes are encapsulated by the

viral proteins forming mature virions. After assembly, the virions are transported to the cell surface in vesicles and released by exocytosis (Fehr & Perlman, 2015).

Possible targets of SARS-CoV-2 (where ACE2 is abundantly present) include the lining epithelium of the lungs, gastrointestinal system, heart, kidneys, and brain. A number of studies are exploring the immune response to SARS-CoV-2, with most suggesting that the immune response occurs by monocyte/macrophage activation, elevation of pro-inflammatory cytokines, depletion of lymphocytes and deployment of large numbers of neutrophils, but the mechanisms involved are not yet well understood(Abdullah & Sharquie, 2020; Domingo *et al.*, 2020; Ezzikouri *et al.*, 2020; Zhand *et al*., 2020).

Understanding the mechanism of coronavirus replication is essential for the development of therapies. As the development of new drugs is a long and expensive process, the knowledge gained from the SARS-CoV and MERS-CoV outbreaks of the early 21st century has helped in the search for treatments against COVID-19, as several interventions cited in the past, with different modes of action, have been studied for the current pandemic. The R&D Blueprint group of the WHO published a document with an overview of experimental treatments for COVID-19, separating the medications according to their classifications (WHO, 2020c).

Some **antiparasitic drugs** have been considered as potential treatments against SARS-CoV-2, such as ivermectin, chloroquine and hydroxychloroquine.

Ivermectin is one of the most important anti-parasitic drugs used in human and veterinary medicine. Some in vitro and in vivo studies (using animal models) indicate the efficacy of ivermectin in inhibiting viral replication through the inhibition of nuclear transport mediated by the α/β1 importin heterodimer(Wagstaff *et al.*, 2012). This inhibition appears to be critical for blocking the replication of different RNA viruses, such as HIV viruses (Wagstaff *et al.*, 2012), Dengue (Tay *et al.*, 2013), Influenza (Götz *et al.*, 2016)and SARS-CoV-2 itself (Caly *et al.*, 2020). To date, the latter cited

study is the only one evaluating the efficacy of ivermectin on SARS-CoV-2 replication in vitro. The results obtained indicated a significant decrease in the levels of isolated viral RNA (originating from the virus) and processed viral RNA (originating from viral replication in host cells) in SARS-CoV-2 infected cells after treatment with 5µM of ivermectin. Furthermore, treatment with different concentrations of ivermectin, in addition to generating a dose- dependent response (IC_{50} ~2µM), did not show significant toxicity levels. Such a result, although unique, encouraged several research groups to immediately initiate clinical trials to evaluate the efficacy and safety of using ivermectin in the treatment of patients with COVID-19 (NCT04422561 and NCT04343092). However, no results are available to date, which is why ANVISA and international bodies such as the FDA conclude that there is insufficient clinical evidence for the use of this treatment and do not recommend the use of ivermectin for the treatment of COVID-19.

Chloroquine is a drug used to treat malaria, lupus erythematosus, rheumatoid arthritis, liver amebiasis, and photosensitive skin rashes. Despite widespread speculation, recent data from large clinical trials have shown no treatment benefit against COVID-19, leading the FDA to withdraw the emergency use authorization issued in March for chloroquine and hydroxychloroquine as treatments for COVID-19.

Some **antivirals** have been raised as alternatives against SARS-CoV-2, such as lopinavir/ritonavir which is mainly used against HIV. Despite limited data to support the use in the treatment of COVID-19, there are an important number of clinical trials registered to test this co-formulation in the treatment against the new coronavirus. Also, remdesivir is a broad-spectrum antiviral that acts as an RNA-dependent RNA polymerase inhibitor, inhibiting virus replication and is therefore an attractive target as a viral inhibitor and considered promising for a future COVID-19 solution. Umifenovir (arbidol) and favipiravir are also antivirals with ongoing studies for COVID-19. Camostat is a serine protease inhibitor approved in Japan for treatment of chronic pancreatitis

due to its ability to inactivate trypsin. This medication has demonstrated inhibitory activity against TMPRSS2, and is therefore a potential treatment drug against COVID-19. Nafamostat is a chemical analog of camostat, but has a very short half-life and may significantly impair clinical utility in SARS-CoV-2 infection.

Biological agents can be great allies in the fight against the new coronavirus. The tocilizumab and sarilumab (kevzara) are recombinant monoclonal antibodies against the IL-6 receptor used for the treatment of cytokine release syndrome, rheumatoid arthritis and systemic juvenile idiopathic arthritis. As patients with COVID-19 also have higher plasma cytokine levels (closely related to disease severity and prognosis), interfering with this condition may also be a possible treatment for severe and critical COVID-19 infections.

Convalescent plasma therapy is another possible alternative, but it relies on the administration of plasma from recovered COVID-19 patients and is more effective as prophylaxis than as treatment, with a better result when administration is done soon after the onset of symptoms.

As **adjuvant/supportive therapy**, there are studies with azithromycin, a macrolide antibiotic, which also has anti-inflammatory and immunomodulatory functions and can minimize the elevated production of cytokines associated with pulmonary viral infections. Also to aid in cytokine production, corticosteroids may be useful in the treatment of severe COVID-19, but they should be used for a limited time and with controlled dose (Chibber et al., 2020; Nitulescu et al., 2020; Zhand et al., 2020).

From the studies already conducted, RENDESIVIR was the first drug officially approved for the treatment of COVID-19, being announced by Anvisa on March 12, 2021. Like AZVUDINE (46T reverse transcriptase inhibitor - NRTI)46T, RENDESIVIR (RNA-dependent RNA polymerase inhibitor) is also a nucleoside analog.

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To establish a standard for the management of patients with COVID-19, the Ministry of Health published a document with guidelines. For **mild cases**, it is important to perform anamnesis, physical examination, and complementary exams (according to availability) for a better approach and management. The physician can proceed with the prescription at his discretion, according to the clinical diagnosis made, observing the local recommendations and the guidelines of the Ministry of Health. For **moderate cases** (who need hospital admission for observation and clinical follow-up, but do not meet severity criteria for admission to the ICU, i.e., do not have organic dysfunctions or hemodynamic instability and do not need mechanical ventilation or other intensive care procedures) hospitalization is recommended until clinical stabilization (absence of fever and dyspnea for at least 48 hours) and improvement of laboratory parameters. For **severe cases**, early therapy and monitoring should be adopted, administering supplemental oxygen immediately to patients with respiratory distress, hypoxemia or shock with target SpO2 92-96%. Systemic corticosteroids should not be routinely administered to treat viral pneumonia or respiratory failure unless otherwise indicated, such as chronic use. Practitioners should be alert to recognize severe hypoxemic respiratory failure when a patient with respiratory distress is failing standard oxygen therapy and provide advanced oxygen/ventilation support. First, noninvasive ventilation should be attempted. According to the Brazilian Association of Intensive Care Medicine, CNAF (high flow nasal catheter) may be tried in dyspneic and hypoxemic patients. If there is no improvement with the use of CNAF, orotracheal intubation is indicated and invasive mechanical ventilation is started. If the patient remains hypoxemic, transfer to a reference hospital for extracorporeal membrane oxygenation should be considered (Ministério da Saúde, 2020c).

AZVUDINE

Nucleoside analog plays an important role in the treatment of antiviral infections. **RNA synthesis is an indispensable step in the coronavirus replication life**

cycle, which is usually successfully performed **using natural nucleosides from host cells as substrates.Nucleoside and nucleotide analog inhibitors are synthetic molecules analogous to purines and pyrimidines, which can be mistakenly incorporated during viral replication, terminating viral RNA chain synthesis or causing mutations that lead to loss of viral viability, preventing viral replication and viral dissemination**(Pruijssers & Denison, 2019).

Nucleoside analogues are successfully used to treat DNA and RNA viral infections, such as HIV, hepatitis B virus, hepatitis C virus, herpes virus, and various respiratory viruses, such as Influenza and the coronaviruses (Carroll & Olsen, 2006; Jordan et al., 2018).

The new coronavirus is a new type of RNA virus.

Several nucleoside analogues have been reported to show effect on inhibition of coronavirus replication in vitro, such as ribarivine, faviparavir and remdesivir, which shows strong effect on inhibition of coronavirus replication in vitro. The aforementioneddrugs are in early stage of research and development, so their safety and clinical efficacy still need to be explored(Choy et al., 2020; Drożdżal et al., 2020; M. Wang et al., 2020). Thus, it is necessary to find new nucleoside analogues for the treatment of COVID-19.

AZVUDINE (FNC) is a new type of nucleoside analog reverse transcriptase inhibitor developed for the treatment of HIV.

Its chemical properties are found in Table 1below:

Chemical name	**4-amino-1-((2R,3S,4R,5R)-5-azido-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1H)-one**

Projeto de Estudo Clínico de AZVUDINE

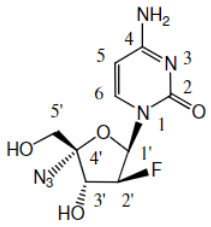
Chemical structure	
Molecular formula	$C_9H_{11}FN_6O_4$
Molecular Weight	286,220g/mol
Properties	Odourless and tasteless, pale yellow crystal

Table1:Chemical properties of AZVUDINE

AZVUDINE is a 4'-C(substituted)-2'-deoxynucleoside (4'sdN) with a 3'-OH group, which mimics the natural deoxynucleotide (dN) and has good stability in acid medium due to its 2'-fluoro substituent. The 4'sdNs retain all the functional groups of a dN, so that HIV replication complexes cannot discriminate between a 4'sdN and dN easily. Once the 4'sdN has been incorporated into the growing DNA chain, it is highly likely that immediate termination of the viral DNA biosynthesis chain occurs, blocking the addition of new dNs (Sun *et al.*, 2020). In vitro studies have shown good antiviral activity of AZVUDINE for both DNA viruses, such as hepatitis B, and RNA viruses, such as hepatitis C and SARS-CoV-2 (R.-R. Wang *et al.*, 2014).

Currently, phase I and II studies with AZVUDINE in HIV-positive volunteers have been completed in China, with good efficacy and safety, and the phase III study is ongoing, also in China. In vitro studies indicate that AZVUDINE has antiviral activity against coronaviruses, and clinical studies with COVID-19 volunteers conducted in China have shown that AZVUDINE has a significant therapeutic effect in the treatment of COVID-19. These data show that AZVUDINE has significant and broad-spectrum inhibition of RNA and DNA viruses, and its clinical safety is good. The clinical trial to

evaluate the efficacy and safety of AZVUDINE was approved by the National Drug Administration (NMPA) for the Phase III clinical study of anti-COVID-19, with approval number 2020L00015.

PRE-CLINICAL STUDIES

In vitro studies conducted in China have demonstrated good activity and selectivity of AZVUDINE in cells infected with SARS-CoV-2. An in vitro activity study demonstrated that the active metabolite CL-236 of AZVUDINE has an antiviral activity against coronavirus.The $EC_{50}$is 1,51µM and the selectivity index is 60 (Tabela 2: CL-236 (metabólito ativo do AZVUDINE) inibe a atividade do coronavírus

). The test results support speculation of the mechanism of action of AZVUDINE against COVID-19.

Compound	EC_{50} (µM)	CC_{50} (µM)	SI
CL-236	1,51	89,94	60
Ribavirin	17,9	>409	>23

Table2:CL-236 (active metabolite of AZVUDINE) inhibits coronavirus activity

Another AZVUDINE in vitro test showed that the active metabolite of AZVUDINE, CL-236, has good antiviral activity against COVID-19 and its EC_{50}against SARS-CoV-2 is about 3.2 µM, which is equivalent to Remdesivir, the positive control drug.

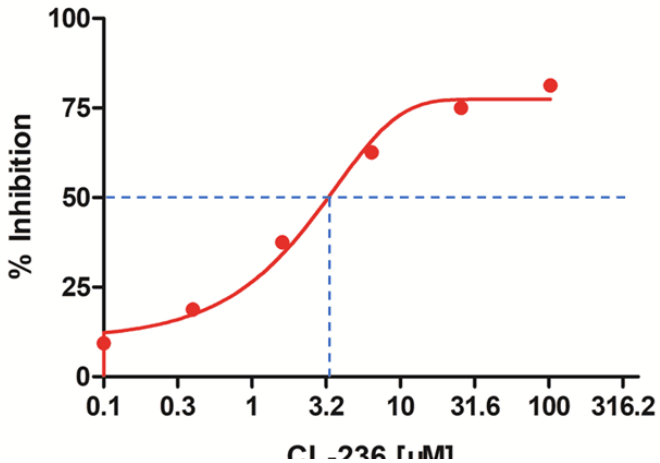


Figure 2: Graph showing the $EC_{50}$of CL-236

In vivo studies have demonstrated the safety of AZVUDINE. Administration of AZVUDINE at single doses of 0.24 mg/Kg, 0.48 mg/Kg or 0.96 mg/Kg had no significant effect on the circulation and respiratorysystem of Beagle dogs. The results of in vivo toxicity testing in Beagle dogs receiving AZVUDINE for 39 weeks showed that at high doses of 0.3 mg/kg, the main target organs are the gastrointestinal tract and the hematopoietic immune system. After 4 weeks of drug withdrawal, the toxic reaction can be basically restored. No toxicity reaction was observed in the 0.03 and 0.1 mg/kg dose groups. Systemic exposure to the drug demonstrated that the level of observable adverse events (NOAEL) is equivalent to 20-30 times the human exposure at the maximum clinical dose used in phase II (4 mg).

Dosages of 1.2 and 2.4 mg/Kg were safe for the nervous system of mice and the dose of 4.8 mg/Kg reduced the sleep time of mice induced by pentobarbital sodium threshold. Sodium pentobarbital is a central depressant with sedative and hypnotic effects. When sodium pentobarbital is administered in threshold doses, drugs that could stimulate or inhibit the central nervous system can increase or decrease the sleep time of mice.

Like many other nucleoside analogues, some dose of AZVUDINE was positive in genotoxicity testing. However, doses more than 1000 times above the clinical dose

caused neither gene mutations by the Ames Test nor chromosomal aberrations in CHL cells in vitro.

No effect of AZVUDINE was observed on mating or fertility, sperm and offspring of male rats. When administered 1.5 mg/kg AZVUDINE in pregnant rats during the perinatal period, slight toxicity was demonstrated, but the offspring rats showed no obvious abnormalities. A small amount of AZVUDINE was detected in milk. The above non-toxic reaction dose is equivalent to 1-3 times the maximum clinical dose in Phase II.

The NOAEL of AZVUDINE for developing progeny rabbits, embryos and fetuses is 1.0 mg/kg, which is equivalent to 5 times the maximum clinical dose in Phase II. Systemic exposure to the drug in rats and rabbits in this study was 50-100 times higher than in humans (the maximum clinical dose in Phase II).

PHASE I AND II CLINICAL STUDIES

Phase I clinical studies showed that there were no serious adverse reactions in the single-dose and multidose trials. Adverse events (AEs) in the single dose trials with 4 mg of AZVUDINE were mainly fever, headache, dizziness, nausea, vomiting and diarrhoea, and the intensity was grade 1.

There were no serious adverse reactions in the multidose trial (GQ-FNC-2014-2), and after continuous administration of AZVUDINE 2 mg, 3 mg or 4 mg for 7 days, HIV- 1 RNA viral load values were significantly reduced (Tabela 3: Resultados do ensaio de multidose de Fase I

Adverse reactions were mainly dizziness and nausea, with grade 1 to 2 severity and no additional treatment was required. No serious adverse reactions occurred in the drug interaction trials (GQ-FNC-103 and GC-FNC-104). The intensity of adverse

reactions in the AZVUDINE, TDF (tenofovir), AZVUDINE + TDF, EFV (efavirenz), and AZVUDINE + EFV groups were all grade 1 and no additional treatment was required.

Group	Decrease value of the viral load
2mg (QD)	1,17 – 2,43 (1,67 ±0,41)
3mg (QD)	0,67 – 1,42 (1,20 ±0,24)
4mg (QD)	0,45 – 2,05 (1,41 ±0,51)

Table3: Results of the Phase I multidose assay

A randomized, multicenter, active-drug-controlled, dose-exploratory, phase II clinical study (GQ-FNC-201) was conducted to evaluate the safety and efficacy of AZVUDINE combined with the antivirals TDF and EFV in HIV-positive volunteers. A total of 172 subjects were included in the study, divided equally into 4 study arms, 3 experimental with doses of 2 mg, 3 mg, or 4 mg of AZVUDINE combined with TDF + EFV and a control with the active drug 3TC combined with TDF + EFV.A total of 172 subjects were included in the study, of which 155 subjects completed the 48 weeks, 15 subjects withdrew from the study due to drug resistance, poor adherence, voluntary substitution with self-pay drugs, AEs, etc. Only 2 individuals withdrew their informed consent forms. In general, subjects had good tolerability and were highly adherent to the treatment regimen and most of the adverse events that occurred were mild or moderate.

After treatment in each group, HIV-1 RNA decreased rapidly. The main indicators of efficacy were the percentage of subjects with HIV-1 RNA <50 copies/mL. The majority of participants' HIV-1 RNA fell below the 50 copies/mL level in 12-24 weeks through 48 weeks. By FAS analysis, in approximately 90% of individuals, from each group, a rate of HIV-1 RNA <50 copies/mL was observed. By the PPS analysis, this

result was observed in approximately 98% of participants in each group. For both analyses, the difference between groups was not statistically significant (P >0.05). A chronological analysis showed that the percentage of subjects with HIV-1 RNA <50 copies/mL in the AZVUDINE 3mg group was slightly higher than at 12 and 24 weeks in the AZVUDINE 2mg, AZVUDINE 4mg, and 3TC groups, and the difference between the AZVUDINE 2mg and AZVUDINE 3mg groups was statistically significant (P <0.05). Among the three AZVUDINE dose groups, the rate of HIV-1 RNA decline in the AZVUDINE 3mg group was relatively faster than in other groups.

The three indicators of secondary outcome assessment were: the change in logarithm of HIV-1 RNA before and after treatment, the number of individuals whose HIV-1 RNA level decreased by ≥1 $\log_{10}$from baseline before and after treatment, and the change in CD4+ T-cell count before and after treatment. After 48 weeks of treatment, changes of approximately -3.2 were observed for each of the groups and there was no significant difference in the decrease in values across groups (P >0.05). The percentage of subjects whose virus RNA level decreased by ≥1 $\log_{10}$was approximately 99%, for each of the groups, and there was no statistically significant difference between the groups (P >0.05). For CD4+ T-cell count, a more pronounced trend towards an increased number of cells could be observed for the AZVUDINE 2mg group, but the analysis of covariance showed no significant difference between the groups (P >0.05).

Protocol (GQ-FNC-201), used doses of 2, 3 and 4mg for **48 weeks and with good safety**. The total incidence of drug-related adverse events (85.7%, 92.9%, 93.0%, respectively) and the degrees of intensity were similar to those in the 3TC group (88.4%). The difference was not statistically significant (P >0.05). Among all adverse reactions, only 3 adverse reactions caused dropout in AZVUDINE 3mg (2.4%), AZVUDINE 4mg (2.3%) and 3TC (4.7%) groups, dizziness, followed by increased ALT, GGT, AST and blood uric acid. The adverse reaction of dizziness was shown to be

slightly higher in grades 1 and 2. Grade 3 adverse reaction occurred only in AZVUDINE 3mg group and 3TC group.The incidence was 2.4% and 4.7%, respectively, and no grade 4 adverse reaction of dizziness occurred. However, the adverse reactions of dizziness that occurred during the study may be related to the drug EFV (Efavirenz).

In summary, AZVUDINE combination treatment with TDF and EFV significantly inhibited HIV-1 RNA replication, and the treatment effect is currently comparable to the first-line treatment plan 3TC + TDF + EFV. The treatment showed good safety, such that the three AZVUDINE dose groups and the 3TC group were similar in the total incidence and severity of adverse reactions.

Recently, on July 21, 2021, AZVUDINE was approved in China by the National Medics Products Administration (NMPA) for the treatment of HIV.

CLINICAL STUDIES WITH COVID-19

FNC-Hope1 was a randomized, open-label, controlled clinical trial to evaluate the safety and efficacy of AZVUDINE tablets in the treatment of COVID-19 conducted at People's Hospital of Guangshan County, China.

Twenty individuals with COVID-19 with mild to moderate symptoms were randomly and equally assigned to 2 groups, experimental and control. Subjects who received conventional treatment with CNF (without antiviral drugs) after diagnosis **reduced the time to nucleic acid negative for the first time by -7,3 days**, compared with those who received conventional treatment alone (9.8). Subjects who received conventional treatment + CNF (without antiviral drugs) **reduced by -6,8 days the time to nucleic acid negative for the first time** compared to those who received only conventional treatment (11,30).

Projeto de Estudo Clínico de AZVUDINE

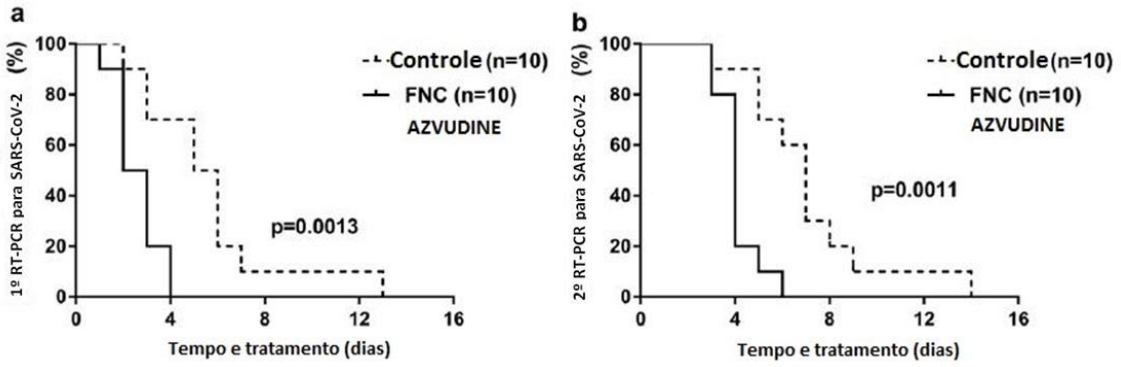


Figure3: Kaplan-Meier curves of two consecutive negative tests for SARS-CoV-2 (a) first and (b) second) in FNC and control group. Data are expressed as percentage (%). Differences between groups were using the Log-rank test (Mantel-Cox)

On the fourth day after treatment, the rate of the first negative nucleic acid conversion reached 100% in patients in the FNC group and 30% in patients in the control group. Kaplan-Meier curves indicate the significant difference (P = 0.0013). On day 6, the negative conversion rate of the second nucleic acid reached 100% in patients of the FNC group and 40% in patients of the control group. Kaplan-Meier curves indicate the significant difference (P = 0.0011).

Regarding safety analysis, **there was no adverse event in the experimentalgroup** and three adverse events in the control group with an incidence of 30% (3/10) during the study period. All adverse events were grade 2 and were followed up until they disappeared. **There were no serious adverse event, serious adverse reactions, major adverse events or death events in the study period.**The demographics and baseline characteristics of the subjects were similar and the proportions between the 2 groups were relatively balanced and comparable.

In 3 other hospitals in China, during the periods from February 14 to March 10, March 1 to March 22 and March 7 to March 30, **32 volunteers with mild to severe symptoms were treated with different antiviral therapeutic approaches**, including Arbidol, Lopinavir/Ritonavir, Interferon, Hydroxychloroquine, Osetalmivir, Ribavirin, traditional Chinese medicine drugs, among others, **before the administration of**

AZVUDINE 5mg was started. Some volunteers received an attack dose of 10mg on thefirst day. After initiation of AZVUDINE treatment concomitant with some different combinations of these antiviral medications, **the coronavirus viral load detection test became negative within 2-10days for 31 volunteers.** One patient reported nausea and vomiting on the first day of AZVUDINE treatment and another patient had **hypokalemia** two days after AZVUDINE administration. Both completed their treatment. One patient dropped out of treatment; however, no further AEs were reported from these and other patients. **There were no serious adverse events (SAEs) or death events during the study period.**

As of January 6, 2021, 209 patients have been enrolled (expected enrollment is 342 patients, for the phase III study - Hope 4 and except for the last 3 patients who entered the group last, all other patients have been cured and discharged.

In terms of safety events, with the exception of individual patients who experienced skin rush or elevated ALT, other adverse events were probably unrelated and no serious adverse events were reported. Up to the first 150 subjects in the random number, there were 45 adverse events in this design, including 31 cases at level 1 and 14 cases at level 2; there were 16 adverse reactions, including 10 cases at level 1 and 6 cases at level 2. The main adverse reactions involved ALT elevation, AST elevation, dermatitis, pruritus and nausea, etc. There were no events or adverse reactions of level 3 or above. With the exception of 2 patients who refused follow-up and 4 patients with a persistent state, all adverse reactions were basically alleviated or even disappeared.

RISKS AND BENEFITS

2.1.1. Risks

The participant's health condition may remain the same, improve or worsen, and there is the possibility of adverse effects occurring during the study. The procedures that will be performed throughout the research period may present some minimal risks

related to the clinic visits, including, but not limited to, possible minor bruising due to blood sampling for laboratory analysis, anxiety due to questioning about health status, and side effects due to the use of the AZVUDINE drug..

Like all medicines, **AZVUDINE may also cause some adverse reactions.** The main reactions reported in preliminary studies at the **4mg** dose **(fever, headache, dizziness, nausea, vomiting and diarrhoea)** were mild to moderate in intensity. Studies with a loading **dose of 10mg and maintenance with 5mg showed a good safety profile**. As the doses may overload the renal and hepatic system, special attention will be given to the exclusion criteria and, for the participants included in the study, there will be monitoring of hepatic and renal function for intervention, if necessary. If even minimal adverse reactions occur, the research team will be available to provide all necessary assistance.

2.1.2. Benefits

There may be no benefits in participating in the study, but in any case, we believe that the search for an effective and safe therapeutic option is fundamental and a priority, so that the conduct of a randomized study along the lines of this proposal is necessary. However, **as this drug has already been shown to be effective in patients with COVID- 19 (with conversion of the viral nucleic acid to negative in a shorter time), it is possible to expect a potential direct benefit to participants receiving AZVUDINE during the study**. Patients in the PLACEBO group will have the assistance recommended for treatment of COVID-19 and all participants will have a follow-up of the disease through monitoring, consultations and examinations.

2.1.3. Analysis of risks and benefits

Phase I and II clinical studies have provided a safety profile for the use of AZVUDINE in HIV-positive volunteers at doses up to 4mg. **In vivo studies have shown safety at doses at least 1000 times higher than those used in the Phase I and II**

studies. Adverse reactions observed with 4mg of AZVUDINE were mainly fever, headache, dizziness, nausea, vomiting and diarrhea, and the intensity of these AEs were mild to moderate.

Preclinical testing for carcinogenesis and reproductive toxicity has shown that the doses used in the clinic are significantly lower than the doses that showed toxic effects in these trials. **No SAE has been reported in clinical trials conducted with AZVUDINE lasting 48 weeks. However, because it is a nucleoside analog, there is a risk for fetal malformation, underscoring the importance of exclusion criteria and follow- up of couples of childbearing age included in the study.**

Preliminary studies conducted with volunteers with COVID-19 used doses of 5 mg of AZVUDINE and some volunteers received the loading dose of 10 mg of AZVUDINE. The data from these studies showed a good safety and efficacy profile compared with patients previously treated with other therapeutic approaches, in which there was no conversion of viral nucleic acid to negative and no improvement in the clinical picture. In volunteers, after AZVUDINE administration, the two consecutive viral nucleic acid tests were negative and all patients were discharged. The AEs reported were mild and disappeared without medical intervention and no SAEs occurred.

It is important to emphasize that the loading doses may overload the renal and hepatic systems due to increased metabolism and excretion of the drug. In view of this **risk, special attention should be given to the eligibility criteria and, for the research participants included in the study, it is important that monitoring of liver and kidney functions be performed in the first days, so that intervention can take place if necessary**.

It is possible to expect a potential direct benefit to participants receiving this drug during the study, the conversion of the viral nucleic acid into a negative one in a shorter time. It is important to point out some risks and benefits linked to the

randomized study model, such as the possibility of the research participant being allocated to the PLACEBO group, i.e., without intervention of the experimental drug, which may be a promising treatment, but is not yet available in clinical practice. However, **participants in the PLACEBO group will have the assistance recommended for treatment of COVID-19 and all participants will have constant monitoring of the progress of the disease through home/outpatient follow-up and examinations.** In addition, participation in a clinical trial requires greater availability of the study participant, since there is a need for frequent follow-up.

There will be a follow-up of the patient at home and on an outpatient basis. Evaluations will be made by trained professionals and any changes will be identified and appropriate actions will be taken to minimize and / or recovery. In addition, the participant will have access and guidance on all the tests performed. **At any time, the participant may have access to all the information obtained about him/her in this study or about the general results of the study after its termination. Withdrawal and/or termination of the study will not affect the medical care offered to the participant, as control visits are scheduled.**

All medical information will be confidential and only the study team will have access to avoid breach of confidentiality. At no time will the participant's name or any information about their health be provided to anyone other than the study team. The information will be confidential and used only for the purpose of this research. All reasonable measures to avoid breaches of participant identity and confidentiality will be enforced. These include access to study documents only for those on the research team, storage of printed documents in a locked file in a location with restricted access, andstorage of electronic data in a database with secure access via individual username and password only for the researchers and other members of the research team.

By proving the clinical efficacy and safety of AZVUDINE, society will be able to benefit from an effective treatment against the new coronavirus.

Projeto de Estudo Clínico de AZVUDINE

JUSTIFICATION

The coronavirus emerged in late 2019, and since then it has been spreading around the world with high speed. In March, the World Health Organization declared a pandemic of the new coronavirus, resulting from the high dissemination of this virus worldwide. Brazil is currently the second country with the highest number of cases, exceeding 200,000 deaths by COVID-19 (WHO, 2020e). As a consequence, the coronavirus has not only led to a large number of deaths until today, but it has also overloaded the health system and also caused impacts on the world and Brazilian economy.

A key strategy in the treatment of COVID-19 would be to find an effective, antiviral agent that leads to a reduction in viral load and, consequently, a reduction in the physiological damage caused by the coronavirus. Clinically approved drugs or drugs in advanced stages of development for the treatment of other viral diseases, such as HIV, should be considered for the treatment of SARS-CoV-2.

AZVUDINE is an antiviral drug that was developed for the treatment of HIV and its safety and clinical efficacy has been demonstrated in HIV-positive patients. The clinical development of this drug is currently in phase III in China. In addition, in vitro studies have shown that AZVUDINE has effective antiviral activity against SARS-CoV-2, and preliminary clinical trials conducted in China have also shown potential efficacy and safety of AZVUDINE in patients with COVID-19.

The number of cases and deaths from COVID-19 has been increasing worldwide and in Brazil in recent months. It is necessary to rapidly find alternative therapies to control the complications of SARS-CoV-2 infection, since the development, immune response, and recovery from the disease caused by this virus are still uncertain.

This study aims to generate rapid, robust, documented evidence on the potential clinical efficacy and safety of AZVUDINE, for patients infected with SARS-CoV- 2.

Projeto de Estudo Clínico de AZVUDINE

3. HYPOTHESIS

AZVUDINE has therapeutic potential and adequate safety profile for the treatment of patients infected with mild stage SARS-CoV-2.

4. OBJECTIVES AND OUTCOMES OF THE STUDY

Primary Objectives	**Primary Outcome**
To evaluate the **efficacy** and **safety** of AZVUDINE (FNC) in patients infected with mild stage SARS-COV-2; [Period: 14 days].	• Proportion of patients hospitalized during the study until day 28, according to the WHO ordinal scale of clinical progression (Jun/2020), Score 4 to 10. [Period: until D15, D21 and D28, approximately 14-30 days.]

Secondary Objective	**Secondary Outcome**
To evaluate the **clinical outcome** of participants infected with mild-stage SARS- CoV-2 treated with AZVUDINE (FNC) versus placebo.	**SECONDARY OUTCOME:** • Proportion of participants with clinical outcome of CURE during the study; [Period: until D15, D21 and D28, approximately 14-30 days]. ○ The clinical outcome of cure is defined in this protocol as the absence of viral RNA in collected samples and clinical conditions for outpatient discharge[4]. • Improvement in clinical status in at least one category compared to screening on the Ordinal Scale of Clinical

[4]According to the WHO Working Group on the Characterization and Clinical Management of COVID-19 Infection (July 2020), the clinical and virological absence of the infection (RT-PCR undetectable) is suggestive of CURE for initially infected participants.

	Improvement (WHO, Jun/2020) [Period: until D15, approximately 14 days]. • Severity and duration of symptoms: fever, cough, fatigue or tiredness, shortness of breath, myalgia, nasal congestion or runny nose, sore throat, headache, chills, nausea, vomiting, anosmia, ageusia 2; [Period: until D15, D21 and D28, approximately 14-30 days] • Changes in liver and renal function baseline; [Period: until D15, D21 and D28, approximately 14-30 days] ○ Laboratory tests performed on days D1, D3, D5, D7, D9, D13, D15, D21, D28 and D60 • Time of AZVUDINE use until the second negative RT-PCR conversion [Period: until D15, approximately 14 days]. • Evaluation of negative conversion time of SARS-CoV-2 viral load by RT-PCR between AZVUDINE (FNC) group and control group; [Period: until D15, D21 and D28, approximately 14-30 days]. • Evaluation of the number of cycles for the detection of viral load of SARS-CoV-2 by RT-PCR and application of the standard curve for calculation of viral load; [Period: until D15, D21 and D28, approximately 14-30 days]. • Analysis of the relationship between the calculated and/or quantified viral load and the clinical evolution of the participants in the AZVUDINE (FNC) group

	compared to the control group; [Period: until D15, D21 and D28, approximately 14-30 days].
	• Occurrence of drug interactions; [Period: until D15, D21 and D28, approximately 14-30 days].
	• All-cause mortality rate during the study; [Period: until D15, D21, D28 and D60, approximately 14-60 days].
	• All-cause mortality rate during the study; [Period: until D15, D21, D28 and D60, approximately 14-60 days].
	• Frequency and intensity of adverse events, unexpected adverse events, and serious adverse events; [Period: until D15, D21, and D28, approximately 14-30 days]
	• Proportion of comorbidity-related worsening [Obesity, diabetes, alcoholism, smoking, lung disease]; [Period: until D15, approximately 14 days].
	• Aggravations occurring in the post-treatment period, up to 60 days or outcome. Period: until D60 [approximately 60 days]
	• To evaluate the tolerability of AZVUDINE (FNC) use at 5mg/day regimen for up to 14 days: [Period: until D15, approximately 14 days].
	  ○ Calculating treatment adherence;
	  ○ Total time of use of AZVUDINE (FNC);
	  ○ Proportion of participants who withdrew consent;

	○ Proportion of participants who abandoned treatment.

JUSTIFICATION OF THE PRIMARY ENDPOINT

According to the document "COVID-19: Developing Drugs and Biological Products for Treatment or Prevention" (FDA, 2020)[5]evaluation of investigational product for the treatment of participants with COVID-19 should adopt as efficacy endpoint, measures that demonstrate clinical significance compared to the control group and the first choice of endpoint for outpatient studies is the proportion of patients hospitalized on day 28, however, participants hospitalized during treatment until day 28 will also be considered.

The ordinal scale was used as a primary and secondary outcome for the assessment of the clinical progression of participants with mild clinical picture over a specific period, where, as recommended, the categories described in the scale should be clearly defined and the corresponding levels should be discrete and non-overlapping (mutually exclusive), to avoid ambiguities in data analysisP5F[6]P.

The WHO Clinical Improvement Ordinal Scale (Jun/2020) is a tool commonly used in clinical trials as a measure of clinical outcomes. On this scale, the WHO Working Group on Characterization and Clinical Management of COVID-19 Infection (July, 2020) presented an 11-point ordinal scale built on literature review and input from international experts in clinical trials, epidemiology, virology, infectious diseases, intensive care medicine, public health, and public policy. This scale was intentionally

[5] FDA. *COVID-19: Developing Drugs and Biological Products for Treatment or Prevention - Guidance for Industry*. USA. Maio/2020. Disponível em: https://www.fda.gov/emergency-preparedness-and-response/mcm-issues/covid-19-related-guidance-documents-industry-fda-staff-and-other-stakeholders

[6] MacKenzie CR, Charlson ME. *Standards for the use of ordinal scales in clinical trials*. Br Med J (Clin Res Ed). 1986;292(6512):40-43. doi:10.1136/bmj.292.6512.40

proposed as a minimum set of ratings, which focuses on the most relevant variables for most participants included in cohort studies or clinical trials. The group also states that the expansion of the categories of this ordinal scale (in relation to the previously published scales, which were 8 points) allows a better distinction of the clinical status of the participants, especially in the lower levels of severity[7].

CLINICAL FRAMEWORK	DESCRIPTION	SCORE
Not infected	Non-infected; no viral RNA detection.	0
Outpatient: mild disease	Asymptomatic; viral RNA detected.	1
	Symptomatic; does not require assistance.	2
	Symptomatic; needs assistance.	3
Hospitalized: moderate illness	Hospitalized; no oxygen therapy *	4
	Hospitalized; oxygen support by mask or nasal catheter.	5
Hospitalized: serious illness	Hospitalized; oxygen support by NIV or high flow.	6
	Intubation and mechanical ventilation, pO2/FiO2 ≥150 ou SpO2/FiO2 ≥200.	7
	Mechanical ventilation pO2/FiO2 <150 (SpO2/FiO2 <200) or	8

[7]John C Marshall *et al*. A minimal common outcome measure set for COVID-19 clinical research. The Lancet Infectious Diseases. Volume 20, Issue 8. (2020): e192-e197. ISSN 1473-3099. https://doi.org/10.1016/S1473-3099(20)30483-7.

CLINICAL FRAMEWORK	DESCRIPTION	SCORE
	vasopressors. Mechanical ventilation pO2/FiO2 <150 and vasopressors, dialysis or ECMO.	9
Death	Death.	10

ECMO: extracorporeal membrane oxygenation; FiO2: fraction of inspired oxygen; NIV: non-invasive ventilation; pO2: partial pressure of oxygen; SpO2: oxygen saturation; *If hospitalized only for isolation, register as an outpatient clinical picture.

Table4: WHO Clinical Progression Scale (Jun/2020))

According to the WHO working group, clinical and virological absence of infection is suggestive of CURE for participants who were initially infected (RT-PCR not detectable) or suggestive of misdiagnosis in inclusion of participants (WHO, 2020).

The maximum treatment time of 14 days respects the average incubation time of SARS-CoV-2 during infection, although previous trials have shown that negative seroconversion of viral RNA detection in participants using AZVUDINE (FNC) can occur within 5 days. In the HOPE-1 study, we observed that two participants in the control group did not have negative seroconversion of viral RNA detection in this period.

5. MATERIAL AND METHOD

STUDY DESIGN

5.1.1. Design

This is a randomized, parallel, double-blind, PLACEBO-controlled clinical trial with 312 participants, who will be randomly assigned to the experimental group and

the control group according to a 1:1 ratio, with 156 in each group, considering a 20% dropout.

In the experimental group, the treatment of participants using AZVUDINE associated with the standard treatment for COVID-19 at the health unit will be observed, and in the control group the standard treatment for COVID-19 at the health unit associated with PLACEBO will be observed.

Table5 belowshows the treatments and dosages:

Grup	Number of Participants	Treatment	Dosage
Experimental group	156	AZVUDINE + Standard treatment	5 mg/day, orally, up to 14 days (1mg/tablet) -
Control group	156	PLACEBO + 1 Standard treatment	5 tablets/day, orally, up to 14 days -

Table5: Study groups

The study consists of 3 periods: **screening, treatment and follow-up.**

In the screening period, patients with mild symptoms suspected of COVID-19 who wish to participate in the study must authorize the collection of nasopharyngeal/oropharyngeal swab samples for RT-PCR analysis by signing the informed consent form. After analyzing the results of the laboratory tests, the investigating physicians evaluated and validated the data that determined the eligibility of the volunteers, and they could start the treatment, after randomization.

In the treatment period, participants randomized to the experimental group will receive the experimental drug **AZVUDINE + standard treatment and those randomized to the control group will receive PLACEBO + standard treatment. Research participants will receive daily oral administration of the investigational products for**

up to 14 days, or until two consecutive negative viral load results are obtained by RT-PCR.

The principal investigator and his team will provide the participants with corresponding treatment measures, according to the clinical symptoms of the individuals, ensuring their safety during the study. Upon completion of the treatment and assessment of clinical symptoms by the investigating physicians, the participant may be discharged from the outpatient clinic.

For **Early Termination (ET)** of treatment, participants should follow the routine of visit 17: End of Treatment (D15), in advance. If two consecutive negative RT- PCR viral load results occur, outpatient discharge may be granted before the end of the 14-day treatment period.

The period between randomization and the end of treatment will be up to 14 days and there may be early termination (ET) or worsening. Participants who escalate to a hospital admission will be discontinued from the investigational drug for several reasons:

1. We don't know which arm the participant is in;
2. Inability to standardize the protocol of conventional treatment and monitoring of each co-participating unit in the period of hospitalization;
3. Even if this participant is in Campos-RJ, where the IGZ-1 study is being conducted, this criterion is not foreseen in the protocol.

However, in case of an aggravation, regardless of the city where the participant aggravates, the cooperation agreements between the Galzu Institute and the management of each co-participating hospital, with the agreement of the city hall/health department, guarantee the hospitalization of the participants, as well as access to data and safety exams, and there is no difficulty in performing safety monitoring in the co-participating units. Therefore, the procedures and exams for

safety and efficacy monitoring in D21 will be performed, as well as the follow-up schedule of D28 and D60, except in case of death.

In the period of follow-up, the participant must return within 28 and 60 days of outpatient discharge for follow-up visits.

The first day of treatment will be day 1 and will last up to 14 days.

Treatment will be terminated early when two consecutive RT-PCR tests are negative (criteria for outpatient discharge) and may be discontinued early in the event of participant dropout, or in the event of SAE.

All participants will undergo efficacy and safety evaluations. Blood samples and respiratory samples (oropharyngeal and nasopharyngeal swabs) will be obtained on days D1, D3, D5, D7, D9, D11, D13 and D14 (home follow up); and day D21 (in case of worsening and hospitalization) and days D28 and D60 (on return to the center for outpatient follow-up). Detailed information about the visit is in item 7.3.

After discharge from the outpatient setting, research participants should follow the instructions below in accordance with the care and attention specified by the research facility:

- Remain in recovery for 3 to 6 weeks;
- Use face masks when necessary;
- Sanitize hands and do not share personal objects;
- Avoid contact with people in the risk group, especially;
- Immediately inform the Principal Investigator or his/her team in case of fever and/or respiratory symptoms.

5.1.2. Justification of the sample size

Based on the article "Hospitalization and mortality associated with SARS-CoV- 2 viral clades in COVID-19"[8], the Hospitalization rate occurred in 18.5% of participants who were infected with SARS-CoV-2. To consider the test drug effective, the researcher adopts the reduction of the hospitalization rate of the intervention group to below 5.5%. That said, using the sample calculation formula for studies with the variable proportion, using the R software and the TrialSize package, we calculated the sample size according to the following command:

TrialSize:Two Sample Proportion.NIS (alpha, beta, p1, p2, k, delta, margin)

Parameters						
alfa	**beta**	**1**	**2**	**k=n1/n2**	**delta=p1-p2**	**Margem**
0.05	.05	.055	.185	1	0.13	0.00

The reduction in the proportion of hospitalizations is based on the open-label, randomized, controlled trial on the efficacy of AZVUDINE 1 mg tablets in the treatment of patients with COVID-19 (HOPE-1)[9].

The reference used for the assumption of this 5.5% rate for the test drug was **adjusted from** the open-label, randomized, controlled **study** on the efficacy of AZVUDINE 1 mg tablets in the treatment of patients with COVID-19 (HOPE-1)[10],as reported below:

- The study included male/female patients aged 18 years and older with a mild course of COVID-19 who received treatment for COVID-19 ("treated") and,

[8]https://doi.org/10.1038/s41598-021-82850-9

[9]Randomized, open, controlled clinical trial of AZVUDINE tablets in the treatment of COVID-19. Study No. FNC-Hope1.Report. – P.-R. China, 2020

[10]Randomized, open, controlled clinical trial of AZVUDINE tablets in the treatment of COVID-19. Study No. FNC-Hope1.Report. – P.-R. China, 2020

patients who did not receive treatment ("newly diagnosed"). All patients received AZVUDINE at a dose of 5 mg per day for 7 days;

- Analysis of this clinical trial shows that at the start of treatment, 100% of patients were tested positive for SARS-CoV-2 RNA, 80% of patients had abnormalities on lung CT and, 50% of patients respiratory symptoms and signs.

- According to the WHO COVID-19 Ordinal Scale of Clinical Improvement, 50% of patients belonged to category 1 and 50% belonged to category 2.

Following on in the report, analysis of individual patient data (regarding relief of symptoms and respiratory signs, body temperature, chest CT scan and testing for SARS- CoV-2 RNA) allowed estimation of individual time to clinical improvement, defined as a decrease in score on the WHO COVID-19 Ordinal Scale of Clinical Improvement in at least one category compared to screening, according to the table below:

PATIENT NO.	**GRUP**	**PATIENT STATUS AT TRIAGE**	**ORDINAL SCALE OF CLINICAL IMPROVEMENT OF THE WHO**		**TIME FROM THE BEGINNING OF TREATMENT TO CLINICAL IMPROVEMENT, DAYS**
			In screening	**7th day of treatment (D7)**	
001	Test group	Treaty	1	0	3
002	Test group	Treaty	2	0	6
003	Test group	Treaty	1	0	4

004	Test group	Treaty	1	0	5
005	Test group	Treaty	2	0	1
020	Test group	Treaty	1	0	5
010	Test group	Newly diagnosed	1	0	4
011	Test group	Newly diagnosed	1	0	5
016	Test group	Newly diagnosed	1	0	5
019	Test group	Newly diagnosed	1	0	6
006	Control group	Treaty	2	0	7
007	Control group	Treaty	2	0	4
008	Control group	Treaty	2	2	8
009	Control group	Treaty	2	0	5
012	Control group	Treaty	1	1	8
013	Control group	Treaty	2	1	6
014	Control group	Newly diagnosed	2	2	14
015	Control group	Newly diagnosed	2	1	7
017	Control	Newly diagnosed	2	1	4

	group				
018	Control group	Newly diagnosed	1	1	14

Based on this report, "improvement in clinical status in at least one category compared to screening" as per the WHO Ordinal Scale of Clinical Improvement occurred in 100% of participants in the test group (10 out of 10) and, 60% in the control group (6 out of 10).

The sample size calculation was performed by means of the software R using the TrialSize package, with the command: Two Sample Proportion. NIS (0.05,0.05,0.055,0.185,1,0.13,0). To estimate the incidence parameter of the primary outcome in the intervention group, due to the absence of studies with the same primary outcome and intervention, we used results of viral load negativation from the article: "A Randomized, Open-Label, Controlled Clinica lTrial of Azvudine Tablets in the Treatment of Mild and Common COVID-19, a Pilot Study".

2.2. Rate of Nucleic Acid Negativity Conversion

The rate of first negative conversion of nucleic acid after 4 d of treatment was 100% in the FNC group and only 30% in the control group (**Figure 2**a). The Kaplan–Meier curves indicated the significant difference between two groups (p = 0.0013). The rate of nucleic acid negative conversion after 6 d of treatment was 100% in the FNC group and 40% in

In this study, the control group had a viral load negativation rate 70% lower than the group treated with Azvudine until the fourth day. Therefore, based on the results of this pilot study and considering that the incidence of the primary outcome in the general population is 18.5%, using the same reduction proportion, the incidence in the intervention group used to calculate sample size was 5.5%, resulting in a sample

size of 130 per group. Adding 20% of dropout, we have a sample size of 312 participants.

The sample size necessary for the IGZ-1 protocol is 260 participants, with 130 participants in each group. Considering a 20% increase to make up for eventual losses of follow-up, we have a total of 312 patients.

5.1.3. Justification for choice of design

In Brazil, due to the logistical difference and, as a strategy, the studies were separated, resulting in the study:

- IGZ-1 for moderate to severe inpatient participants and;
- IGZ-2 (this study) for mild stage participants on a home-based/outpatient basis.

The choice of phase III design was inspired by the HOPE 1 study, where the therapeutic regimen proposed in this study is based on preliminary studies conducted in China in patients with pneumonia caused by SARS-CoV-2. **The dose used was 5mg, and the AEs observed were mild to moderate, with no need for medical intervention.**

For this study, **the proposed treatment period is 14 days**, in view of the average incubation time of coronavirus and the time of use of AZVUDINE that **in preliminary studies, ranged from 2 to 12 days.**

Evidence from Hope I, discussed above, demonstrates that individuals receiving conventional treatment + CNF (without antiviral drugs) after diagnosis **had a-7,3-day reduction in the time to nucleic acid negative for the first time** compared with those receiving conventional treatment alone (9.8). Subjects who received conventional treatment + CNF (without antiviral drugs) **reduced by -6,8 days the time to nucleic acid negative for the second time** compared to those who received only conventional treatment (11,30).

Projeto de Estudo Clínico de AZVUDINE

In the phase III - Hope 4 study with 342 participants, 209 participants, except for the last 3 patients who entered the group last in January 2021, all other patients were cured and discharged. In terms of safety events, except for individual patients who had skin rash or elevated ALT, other adverse events were probably unrelated and no serious adverse events were reported. There were no serious adverse events, serious adverse reactions, major adverse events or death events in the study period.

Recently (2020), the journal NATURE published the article AZVUDINE (FNC): a promising clinical candidate for COVID-19 treatment"[11], which considers AZVUDINE a promising candidate for the treatment of COVID19, demonstrating its potential in inhibiting viral replication.

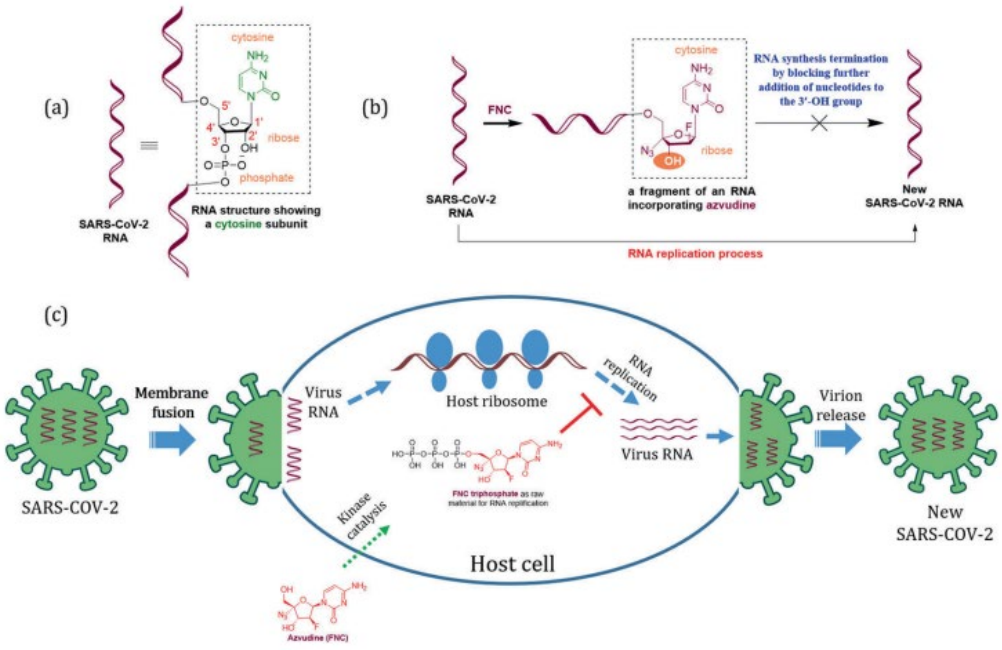


Figure4: Mechanism of action of AZVUDINE a) Azvudine nucleoside-based anti-HIV clinical candidate (FNC) for the treatment of COVID-19. Structure of SARS-COV-2 RNA showing a cytosine subunit; b) Diagram showing the RNA replication process of SARS-COV-2 blocked by FNC; c) Proposed mechanisms of FNC to inhibit SARS-COV-2 activity.

[11]Yu, B., Chang, J. AZVUDINE (FNC): a promising clinical candidate for COVID-19 treatment. Sig Transduct Target Ther 5, 236 (2020).
https://doi.org/10.1038/s41392-020-00351-z (https://www.nature.com/articles/s41392-020-00351-z)

To minimize study bias, research participants will be randomized and treatment will be administered blinded by both research site staff and participants. The research site's standard treatment will be used to avoid any complications/aggravations of the disease and PLACEBO will be used for masking purposes only.

5.1.4. Rationale for dose choice

The dose of AZVUDINE that will be used in this study is the same as that used previously in exploratory studies in China to treat patients as COVID-19. The investigational drug in question **is a new type of nucleoside analog reverse transcriptase inhibitor that is showing good clinical efficacy in treating patients with SARS-CoV-2 infection in China.**

Currently, the phase III study (FNC-Hope4), with 342 participants, has 215 participants, where the dose used was 5mg/day for up to 14 days, with no EAG reported. A partial and confidential report was issued to ANVISA (ANNEX 5).

The therapeutic regimen proposed in this study is based on preliminary studies conducted in China in 41 patients with pneumonia caused by SARS-CoV-2. **The dose used was 5mg/day, and the AEs observed were mild to moderate, with no need for medical intervention.** All patients were completely discharged from hospital after two consecutive RT-PCR tests. Of these 41 patients, 8 volunteers used an attack dose of 10mg on day 1 and 5mg on subsequent days. **The final report (FNC-Hope1) showed benefit to participants treated with AZVUDINE, while in other therapeutic approaches they had no improvement of the clinical picture and nucleic acid detection did not turn negative.**

Regarding the dose-related safety profile used in humans, the phase I dose explorer clinical study (GQ-FNC-2014), evaluated the safety of AZVUDINE in HIV-positive patients for 7 days and demonstrated that AZVUDINE has clinical safety. The

groups with AZVUDINE dose regimen to 2mg, 3mg, 4mg and 5mg showed good safety. No AEs were reported in this study and no participants in the AZVUDINE 3mg and 5mg groups reported any AEs. No serious adverse events were attributed to AZVUDINE.

In this trial, **the plasma concentration of AZVUDINE in patients receiving a 5 mg dose was approximately 2.42ng/mL**, which is much lower than the plasma exposure level of the drug at the NOAEL dose (5.6 mg) in animals ($C_{máx}$ in mice: 164-210.27 ng/mL, $C_{máx}$ in dogs: 85.84-102.55 ng/mL) and 3.099 times lower concentration the dose used in the chromosome structure aberration test of CHL cells with the concentration of 7.5 μg/mL after 4-hour exposure with or without S9 metabolic activation system.

In this same trial, volunteers receiving 3mg and 4mg of AZVUDINE in a single dose had a plasma exposure of approximately 2.85 and 3.4 ng/mL, respectively. A AUC_{0-t}increased gradually in the 3mg, 4mg and 5mg groups when compared to the 2mg group, with a statistically significant difference between the 4mg and 3mg groups.

Pharmacokinetic data suggest that FNC is rapidly absorbed and slowly eliminated. Total urinary excretion of AZVUDINE increases with increasing dose. Excretion between 0-12h accounted for more than 70% of total excretion in 0-24h.

For the 5mg dose of AZVUDINE, the mean half-life is 13.8h, being excreted in the urine in up to 24h.

Protocol (GQ-FNC-201), used doses of 2, 3 and 4mg for **48 weeks with good safety.** In the exploratory clinical trials conducted in China, a higher than safety-verified dose (5mg) was used with a dosing regimen of "On Day 1 10mg" in 8 cases at Henan Provincial People's Hospital and yet there were no serious adverse events, serious adverse reactions, major adverse events or death events in the study period. The current dosing design does not use the first dose of 10 mg and the current ongoing Phase III clinical trial in China, the dosage is "5mg daily".

Projeto de Estudo Clínico de AZVUDINE

In vivo toxicity results from multiple doses of FNC at high dose in rats (human equivalent 11.2-56 mg) and dogs (human equivalent 11.16-37.20 mg).

Preclinical trials evaluated administration times much longer than suggested in this clinical trial, leading to a total equivalent human dose used in these trials of 116.6 mg (1 month) to 1,088.10 mg (39 weeks).

Animal / human equivalent dose	Animal / Administration time	Total dose used in pre-clinical tests over time
3,72mg	**1 month**	116,6mg
5,60mg	**3 months**	504,0mg
3,36mg	**6.5 months (26 weeks)**	655,2mg
3,72mg	**9.75 months (39 weeks)**	1.088,1mg

Table6: Relationship of dose and administration time

The **maximum dose of AZVUDINE proposed for this clinical trial is UP to 70 mg in UP to 14 DAYS, remembering that the participant may be discharged earlier, reducing the total dose.** Long-term drug exposure has demonstrated that **observable adverse events appear when the dose is exceeded 20-30-fold** in animals.

Considering that the maximum safety dose study for AZVUDINE was performed for 5 mg, a daily dose of 5 mg may meet the clinical treatment. The proposed dose of 5mg in the clinical trials for COVID-19 is slightly lower than the lowest NOAEL dose in the fertility and early embryo developmental toxicity trials (5.6 mg).

Based on these studies, there is increasing evidence that the 5mg dose may have clinical efficacy against SARS-CoV-2 infection and its safety profile is compatible with use in the proposed target population.

5.1.5. Place where the research was carried out

The proponent institution will be the Clinical Research Unit of the High Complexity Center / department of clinical research / Galzu Institute, since it has the entire structure of personnel management, molecular biology laboratory for the centralization of sample analysis, daily medical monitoring focused on pharmacovigilance, its own software for real-time data collection, which can be made available to the co-participating units (safety, confidentiality and data quality, control of dispensing/accountability of the experimental drug), in addition to quality management tools.

The co-participating units are hospitals in the northern region of the state of Rio de Janeiro, represented by a member of its clinical staff, which has a very well-structured family health structure (due to oil royalties) and, at the time of the pandemic, was transformed into a service with home monitoring, outpatient care with hospital support in case of worsening. In this case, the prospection will occur in the outpatient clinics of the hospital structure, UPAs and UPHs, by multidisciplinary teams hired to monitor the participant, collect samples for tests, dispense the drug every 48 hours, home/outpatient monitoring, among other activities.

The Researcher Responsible for the study is the same as that of the Proponent Institution. Each co-participating institution acts in the execution of the project, according to the part that is its responsibility, under the responsibility of the sub-investigator, just like the proponent, and according to LETTER Nº 0212/CONEP/CNS the co-participating institutions, participate in the execution of the project just like the proponent, despite not having proposed it, signing:

- Letter from the co-participating institution of awareness and authorization from the Institutional Head;
- Declaration of infrastructure for the safe conduct of research on its premises;

- Declaration of reading and agreement with the ethical opinion of the proposing institution.

The role of the investigator and sub-investigator is addressed in the RDC Nº 9 of 20 February 2015 and in the ICH Good Clinical Practice Guide(E6), where obligations and responsibilities of the sponsor and investigator are not confused, as well as the responsibility for coordinating the trial and ensuring compliance with good clinical practice.

The Research Unit of the CAC was qualified by the Study Sponsor in compliance with the standards established by the ICH Guidelines for Good Clinical Practice GCP/ICH- ICH Topic E6 (R2) (2016), Normative Instruction nPoP. 20, 2017 of ANVISA, whichnprovides for the inspection procedures in Good Clinical Practices for Clinical Trials with Investigational Medicines and other applicable regulations.

The choice occurred due to the infrastructure necessary for outpatient care / monitoring of participants and optimization of the logistics of molecular biology analysis. Centralizing the analyses in the Molecular Biology Laboratory were strategic decisions to maintain the quality of the process in the proof of concept (we will explain later).

Renowned national research institutes, such as FIOCRUZ, use the strategy of multicenter models in their studies. However, in studies with the same design profile, the participating centers are all in metropolitan areas of Rio de Janeiro, most concentrated in the municipality itself. This strategy certainly favors the capture strategy, but does not bring great contributions to evaluations of the influence of demographic or epidemiological profiles on the data of the studies.

TRIAL POPULATION

5.1.6. **Recruitment and Selection**

The research center is responsible for defining the strategies for recruitment and initial screening of research participants, therefore, the **recruitment strategy will**

be the health network of each co-participating unit, since patients with symptoms of COVID-19 in mild phase end up seeking care from the units and Centers for Combating COVID19 (strategy used in the state of Rio de Janeiro).

The fundraising strategy provides for the attraction of volunteers, through agents of prospection, throughout the region of Norte Fluminense, in addition to patients who seek care locally. The fundraising also involves partnerships with the Health Secretariats of the North and Northwest Fluminense region with their municipalities, which do not have a robust infrastructure for hospital care.

Municipalities: Campos dos Goytacazes, Cardoso Moreira, São Fidélis, São Francisco de Itabapoana, São João da Barra, Carapebus, Conceição de Macabu, Macaé e Quissamã, do norte fluminense; e Itaperuna, Bom Jesus do Itabapoana, Italva, Laje do Muriaé, Natividade, Porciúncula, Varre-Sai, Santo Antônio de Pádua, Aperibé, Cambuci, Itaocara, Miracema and São José de Ubá, fron the Fluminense northwest.

After screening with the nursing staff of the health units, axillary temperature, blood pressure, pulse oximetry, heart rate, respiratory rate and presence of risk factors (such as age over 65 years, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, lung disease, immunosuppression, cancer or use of immunosuppressive medications) **are checked.**

The evaluation of symptomatic patients without evidence of viral pneumonia or hypoxia (Sat O2 < 95%) seeks the identification of the signs and symptoms below [12], [Score 1-3]:

MOST COMMON SYMPTOMS	NONSPECIFIC SYMPTOMS	NEUROLOGICAL MANIFESTATIONS

[12]OMS, Clinical management of COVID-19: living guidance, 25 January 2021, Table 6.1, p.19.

Fever (83-99%)	Sore throat Nasal	Dizziness, Agitation,
Cough (59-82%)	congestion	Weakness,
Fatigue (44-70%)	Headache, diarrhea	Seizures,
Anorexia (40-84%)	Nausea	Problems with speech or
Shortness of breath (31-	Vomiting	vision,
40%)	Loss of sense of smell	Sensory loss or problems
Myalgia (11-35 %)	(anosmia)	with balance in standing or
	Loss of taste (ageusia)	walking.

If contamination is suspected, a rapid test for the diagnosis of COVID-19 **will be performed and**, if the test result is positive, the clinical study will be presented by the prospection team and an invitation to participate in the study will be extended. The consent process will be conducted by the prospecting agents in the field, duly delegated and trained to conduct the process, as well as to apply the Informed Consent Form (ICF). Multimedia resources will be used to clarify and instruct the volunteer about the objectives and details of the study. At the end of all the necessary clarifications, the volunteer will be asked to sign the ICF, where one copy will remain with the volunteer and another will be destined to the research center.

If the patient accepts to be a volunteer in the study, he/she and his/her companion, if applicable, will be informed of the study procedures, including laboratory tests in the screening period, the criteria for inclusion and exclusion from the study, his/her rights and duties, the period of treatment and follow-up. **The eligibility criteria may be explored after the anamnesis, performance of laboratory tests but may also be suspended at any time before the complete evaluation if exclusions are identified by the study team.** After this phase, the participant will be **invited to sign the ICF** and, only after signing, the volunteer will be included in the study. Clinical and laboratory evaluations and collection of nasopharyngeal and

oropharyngeal swab specimens for RT- PCR testing may **confirm study participant eligibility once all screening values are available.**

Research participants who meet the inclusion criteria and do not meet the exclusion criteria, and who have eligibility assessed by the Principal Investigator, will be eligible.

Once eligible, the **volunteer will then be referred to the co-participating unit**, for home/ambulatory follow-up, being **immediately randomized, and** starting to use the experimental/placebo treatment, in addition to the standard treatment, following the Treatment Protocol for COVID- 19 of the Guidelines for diagnosis and treatment of COVID-19, Version 1, April 6, 2020, Secretariat of Science, Technology, Innovation and Strategic Health Inputs - SCTIE (ANNEX1):

> *Initial evaluation by checking axillary temperature, blood pressure, pulse oximetry, heart rate, respiratory rate and presence of risk factors (such as age over 65 years, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, lung disease, immunosuppression, cancer or use of immunosuppressive medications). Know if the patient has had contact with someone with suspected or confirmed COVID-19 and if they work in any health care facility or provide patient care.*
>
> *Adoption of symptomatic treatment, home support measures (mild stage) should guide the use of mask, isolation and outpatient follow-up care and if there is worsening.*

5.1.7. **Screening**

The research participants will be screened following the inclusion and exclusion

criteria.

5.1.8. **Inclusion criteria**

Individuals who present the following characteristics will be included in this study:

1. Age ≥18 years, regardless of gender;
2. Fluorescence RT-PCR test result of respiratory or blood samples must be positive for COVID-19, or viral gene sequencing of respiratory tract samples must be highly homologous to COVID-19; Individuals with COVID-19 must meet the diagnostic criteria in the "latest version of clinical guidelines for COVID-19" issued by the World Health Organization (WHO) on June 4, 2020;
3. Symptomatic patients who meet the WHO case definition for COVID-19 without evidence of viral pneumonia or hypoxia (Sat O2 < 95%)[13]P. [score 1-3].
4. Have signed the informed consent form.

5.1.9. **Exclusion criteria**

Individuals who present one or more of the following characteristics will not be eligible to participate in this study:

1. Know or suspect that you are allergic to any of the components of AZVUDINE tablets (inactive ingredients: microcrystalline cellulose, lactose hydrate, polyvinylpyrrolidone K30, croscarmellose sodium, magnesium stearate);
2. Individual presenting shortness of breath and Sat O2 < 95%; or any other symptom requiring treatment through hospital admission;
3. Patients with liver disease (total bilirubin ≥2mg/dL, ALT/TGP and AST/TGO ≥5 times

[13]Signs and symptoms presenting with COVID-19: Most people present with fever (83-99%), cough (59-82%), fatigue (44-70%), anorexia (40-84%), shortness of breath (31-40%), myalgias (11-35%). Nonspecific symptoms: sore throat, nasal congestion, headache, diarrhea, nausea, vomiting, loss of smell (anosmia) and loss of taste (ageusia). Neurological manifestations include: dizziness, agitation, weakness, seizures, problems with speech or vision, sensory loss, or problems with balance in standing or walking. WHO, Clinical management of COVID-19: living guidance, 25 January 2021, Table 6.1, p.19.

above normal limit);P9F[14]

4. Patients with a history of known liver disease (cirrhosis with ChildPugh classification B and C);
5. Patients with a history of renal insufficiency (glomerular filtration rate≤60mL/min/1,73mP^{2}P);
6. Patients with a history of congestive heart failure (NYHA grade 3/4), untreated symptomatic arrhythmias or myocardial infarction within 6 months;
7. Individuals with malabsorption syndrome, or other conditions affecting gastrointestinal absorption, and circumstances in which patients require intravenous nutrition, or cannot take medications orally or nasogastrically;
8. Total neutrophil count <750 cells/L;
9. Women who are pregnant or lactating, or of childbearing potential during the study period and within 6 months after termination of administration;
10. Patient who have participated in another clinical trial or used any experimental drug within the last 12 weeks;
11. Patient under treatment for HIV, Hepatitis C, active Hepatitis B;
12. Patients being treated with other antivirals (e.g., lopinavir/ritonavir, remdesivir, umifenovir/arbidol, favipiravir, interferon-α) or immunosuppressive medications for other medical conditions;
13. Patients under treatment with monoclonal antibodies (Ex: tocilizumab and sarilumab/kevzara);
14. Any clinically significant medical condition or medical history that, in the investigator's opinion, may discourage study participation, such as corrected QT interval >480 on electrocardiogram, among other conditions.

5.1.10. **Follow-up**

[14] Normal values: BT up to 1.2 mg/dL; TGO Adult 12 to 46 U/L; TGP Adult 03 to 50 U/L; GGT Man 10 to 50 U/L, Woman 07 to 32 U/L (Source: Instituto Hermes Pardini).

Follow-up will be done at home during the contagious phase and at outpatient clinics after the negative conversion of the second RT-PCR test. The field agents will perform:

- They will dispense the medication every 48 hours;
- Collection of samples for testing every 48 hours, according to schedule;
- Orientation of the family regarding prevention and isolation measures;
- Scheduling of outpatient visits;

VIROLOGICAL DIAGNOSIS

5.1.11. Diagnostic criteria for SARS-CoV-2 infection

Diagnostic criteria will be based on the World Health Organization Guide, where confirmed cases of SARS-CoV-2 infection will be those performed by molecular testing with viral sequencing by QUALITATIVE real-time PCR/ RT-PCR(WHO, 2020a)of nasopharyngeal and oropharyngeal swab specimens, bronchoalveolar lavage, nasopharyngeal tracheal aspirate, blood, serum and tissue from lung biopsies.

5.1.12. Process standardization at proof of concept

UAccording to the protocol of the IGZ-2 study, RT-PCR examinations will be performed every 48h,Uand as we will probably have patient inflow on even and odd days, processing should be done every day, with results every 24h.

Therefore, the prompt Udavailability of RT-PCRU test results impacts the assessment of eligibility, total treatment time and early termination of participant's treatment.We know that RT-PCR can demonstrate the negative Uconversion of the presence of virus copiesU (QUALITATIVE analysis), while a ddPCR can determine the Unnumber of virus copiesU (QUANTITATIVE analysis).

We also know that U It is possible to calculate the amount of viral RNA copiesU by means of a standard curve to quantitatively determine the viral load of an unknown sample from samples with known amounts.

> *Wang et al, J Clin Invest. 2020 demonstraram que os valores de CT observados nas amostras respiratórias de pacientes críticos foram inferiores (18-22) aos observados em pacientes leves (32-38). Portanto, quanto menor a CT, maior a quantidade de RNA viral na amostra e consequentemente maior a carga viral do voluntário.*

Based on these variables, we Upadronized the process to quantify the viral load as described below, since **very few studies relate the number of cycles with viral loador with the efficacy of the experimental drug. This is a little explored problem that may bring to light issues of virus behavior in relation to clinical response, already questioned by the FDA.**

According to FDA guidance "COVID-19: Development of Biologic Drugs and Products for Treatment or Prevention - Guidance for Industry; 2020," "... virologic endpoints are not appropriate as primary endpoints in a phase III study **because there is no established predictive relationship between the magnitude and timing of viral reductions and the extent of clinical benefit of how a patient feels, functions, or survives."**

THE PROBLEM

UEach RT-PCR equipment has its own sensitivity and different RT-PCR reagent kits have different performances, according to the technical note of May 2020 "ANVISA - ACCURACY OF DIAGNOSTIC TESTS REGISTERED IN ANVISA for COVID-19 for qualitativeanalyses".

Projeto de Estudo Clínico de AZVUDINE

UA sensitivity of each RT-PCR equipment and kit used varies in the number of CTs to generate the qualitative result (Positive or negative)UP10F[15]PU.

Therefore, to extract **RELIABLE QUANTITATIVE** data, both the equipment and the reagent kit must be standardizedU to avoid discrepancies. However, this is not enough, so a standard curve should provide the correlation between CTs and viral RNA copy number.

In this way, we will use the QuantStudio5 RT-PCR equipment, Applied Biosystems, NS: 272525086, ANVISA Reg.: 10358940069, UReg. ANVISA: 10358940069,to issue reports for statistical accounting and to assist the standard curve analysis, via the dedicated softwareP11F[16]P. We will also use the TaqPathP™PCOVID-19 CE-IVD RT-PCRP12F[17]Pkit (UReg. ANVISA: 10358940107),Uwhich can provide us the values of CTs related to viral load (viral RNA copies) as a function of the standard curve constructed with the positive control (TaqPath COVID-19 Control), which is the viral RNA of SARS-CoV-2 at a known concentration of 1 x 10P4P copies/µL. By means of the standard curve, using serial dilutions of the positive control, we can arrive at the concentration (in number of copies) of the viral RNA present in each sample of the volunteers and participants.

> *For example, it is common to determine the concentration of proteins in a sample using a colorimetric method, the Bradford assay. Similarly, by performing serial dilutions of the positive control we can establish a standard curve (equation y = ax + b) using the CTs obtained for each point on the concentration curve.*

[15]KiCqStartTM One-Step Probe RT-qPCR ReadyMixTM. Catalog Number KCQS07, KCQS08, KCQS09. SIGMA-ALDRICH
2019-nCov CDC Probe and Primer Kit for SARS-CoV-2 CERTIFICATE OF ANALYSIS. Catalognumber: KIT-nCoV-PP1-1000. Bioresearch Technologies.
2019-nCoV TaqMan RT-PCR Kit. Catalognumber:TM67100, TM67120. NORGEN BIOTEK COPORATION.
[16]Real-time PCR handbook, 2012. Basis of Real-time PCR, Life Technologies. Inc. CO32085 0812.
[17]TaqPathTM COVID-19 CE-IVD RT-PCR Kit INSTRUCTIONS FOR USE. Catalog Number A48067 Publication Number MAN0019215.

The positive RT-PCR result occurs in CTs ≤37. In this case, when viral RNA is present, the specific probe used for detection of SARS-CoV-2 is broken down by DNA polymerase, emitting fluorescence. The higher the viral RNA copy number, the greater the fluorescence, and therefore the CT value appears earlier during the reaction.

The lower the viral RNA copy number, the lower the fluorescence and consequently the CT value appears later. CT values >37 are considered negative. By establishing a curve of viral RNA concentration (present in the positive control), we will obtain a curve of CTs, from lower values (higher viral RNA copies) to higher values (lower viral RNA copies).

The trials approved for the treatment of COVID19 to date use RT-PCR negative conversion monitoring as the endpoint, whereas the IGZ-2 assay methodology, in addition to obtaining a positive or negative result for the presence of SARS-CoV-2, can also calculate the number of viral copies in each sample and in a practical manner:

- It would be possible to determine the behavior of the viral load after the use of the experimental drug and the placebo;
- It would be possible to relate the different viral loads of the positive result with the clinical improvement over time, individually and globally, in both groups;
- It would be possible to observe the scale of growth (exponential or logarithmic) and the best moment of intervention.

In a positive test, there is an expressive range of variability of viral RNA copies that may be related to the clinical condition of the participant and, since viral load monitoring will be done every 48h, an algorithm can be reached to explain why positive people have different outcomes.

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MEASURES TO MINIMISE STUDY BIAS

5.1.13. **Randomization**

The research participants, according to eligibility at the time of screening and agreement of the Principal Investigator, will be randomized to one of the two arms of the study (1:1) by the Research-IGZ 2.0 software, generating via system, random numbers that relate the groups to the volunteers, from the data management plan of **ANNEX 2**.

5.1.14. **Blinding**

A physical report of the blinding will be generated as a safety measure and sent to the pharmacist for dispensing the investigational product and to the site coordinator in a sealed envelope. Details on blinding can be found in the Randomization topic in **ANNEX 2.**

5.1.15. **Breaking of the emergency blinding**

The breaking of the EMERGENCY BLIND can be **INDIVIDUAL or TOTAL**, via system through the administrator password of the Research-IGZ 2.0 software.

Breaking of blinding must be justified by the Principal Investigator, and it is certain that any breach of blinding will generate Logs in the system, which may be seen in the audit analyses. In case of unblinding, the Principal Investigator and the center coordinator will opt for INDIVIDUAL unblinding. Only in recurring SAEs, suspected of being related to the investigational product, will there be the breaking of the TOTAL blinding.

5.1.16. **Rules for breaking the blinding**

This study adopts double-blind method:

Primary disclosure will be performed after completion of the final version of the statistical plan, audit report and database lock. At this stage, random numbers will be grouped according to the study arm for statistical analysis by group.

Secondary disclosure discloses the drug corresponding to the group code, investigational drug or PLACEBO. The disclosure documents will be signed by the Sponsor and Research Coordinator.

6. TREATMENT OF PARTICIPANTS

For the purposes of this protocol, AZVUDINE is the investigational medicine that will be administered concomitantly with the standard treatment for COVID-19, as per healthcare organisations' guidelines. The investigational medicine will be compared to PLACEBO, also administered concomitantly with the standard treatment for COVID-19. When referring to the investigational (AZVUDINE) or control (PLACEBO) product, the term investigational medicinal product or PLACEBO will be used.

STANDARD TREATMENT GIVEN IN THE CARE OF PATIENTS WITH COVID-19

The Principal Investigator and his/her team should follow the research site's own COVID-19 treatment protocol as described below, providing participants with treatment measures consistent with the individuals' clinical symptoms and recommended by the Ministry of Health (MS/SCTIE, 2020).

The **research participants who evolve to moderate symptoms** should be referred to hospital care, and it is certain that in case of hospitalization, they should discontinue the experimental drug, since the follow-up in the hospital unit requires a study project of its own; however, those aggravated will be followed up during their hospitalization until the outcome.

Mild cases should be managed with non-pharmacological measures such as rest, hydration, adequate food, besides analgesics and antipyretics and home isolation for 14 days from the date of onset of symptoms.

The standard care protocol indicates the use of some drugs such as chloroquine or hydroxychloroquine, oseltamivir, anticoagulants, corticosteroids, with specific instructions for each drug. However, specific recommendations should be made to the team involved in the study, so that there is no administration of drugs prohibited in this study protocol.

EXPERIMENTAL TREATMENT

6.1.1. **Formulation and appearance**

AZVUDINE: The lyophilized formulation of AZVUDINE is a preservative-free, white to yellowish-white, odorless, tasteless solid containing 1mg of AZVUDINE.

AZVUDINE is supplied as a sterile product in a white, round, wide mouth, high density polyethylene vial with a polypropylene cap. In addition to the active ingredient, the lyophilized formulation of AZVUDINE contains the following inactive ingredients: microcrystalline cellulose, hydrated lactose, polyvinylpyrrolidone K30, croscarmellose sodium, magnesium stearate.

PLACEBO: The manufacturing and packaging process for PLACEBO is the same as for the investigational medicinal product, conducted by the same manufacturer and production line. The lyophilized PLACEBO formulation provided is identical in physical appearance to the active lyophilized formulation and contains the same inactive ingredients and will be used in the study for masking purposes.

6.1.2. **Packaging and labelling**

The investigational drug and PLACEBO will be shipped by the sponsor to the research site in white, round, wide-mouthed, high-density polyethylene vials with country-specific labeling and polypropylene caps.

Projeto de Estudo Clínico de AZVUDINE

AZVUDINEtablet 1mg / PLACEBO

35 tablets / bottle

FOR CLINICAL RESEARCH ONLY

Internal Use / Prescription in accordance with the Research Protocol

SEARCH CODE: IGZ-2

Producer: "Beijing Union Pharmaceutical Factory" China.

Address:No. 37, Yongwang Road, Bio-medicine Industry Park, Daxing District, Beijing, China.

ORPC/CRO: Galzu Institute for research, teaching, science and applied technology.

Name of the Principal Investigator: Carlos Augusto de Araujo Tavares.

Lot Nº: _____ Date manufactured: _____

Patient randomization Nº_____

(01)02003659821451(30)1200

Keep out of reach of children

Maintain at room temperature

Validity: 2 years

Figure5: Label for clinical research medicine

Investigational products will be received by the center in separate boxes of AZVUDINE and PLACEBO, with serial number from "X to Y" for AZVUDINE and serial number from "Z to W" for PLACEBO, for example.

There will be a label for participant identification during dispensing, which will be done by double checking. Each vial with 35 individual tablets will have a label with the information in Figura 6name and address of the manufacturer, lot number, medication identification number, participant information, care statement, instructions

for use and dosage (mg), expiration date, storage conditions, and an indication of "For clinical trial use ONLY".

All study medications will be prepared, packaged and labeled in accordance with the standard operating procedures (SOPs) of the sponsor or its representative, Good Manufacturing Practice (GMP) guidelines, International Council on Harmonization (ICH) Good Clinical Practice (GCP) guidelines and applicable national regulations. All labels on the packaging will comply with local regulations.

6.1.3. **Storage and stability**

The shelf life of AZVUDINE and PLACEBO is 2 years. The storage of the investigational medicine and PLACEBO should be at room temperature in a dark place.

The product proved stable in high temperature and humidity tests at 40°C and 60°C and at 92.5% RH in closed storage and, for up to 30 days, no significant changes were observed in the stability tests of the drug to be used in clinical trials in Brazil. The samples stored open at 75% RH, on the 30th day, presented humidity absorption of more than 5%.

6.1.4. **Dose and method of administration**

Research participants will be randomized 1:1 to receive either the experimental medication or PLACEBO. AZVUDINE or PLACEBO will be provided to the participant initially at this first outpatient visit and every 48 hours thereafter during home visits for testing by a qualified research team member delegated for this role. The administration, date and time, will be entered in the study eCRF.

Therefore, AZVUDINE will be administered orally at a dose of 5mg, once a day, while the participant is on outpatient treatment, for up to 14 days of the total course or until two consecutive negative viral load tests by RT-PCR are obtained.

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The research participants will be instructed to swallow the medication whole, without breaking, chewing, crushing or dividing it before ingesting it. The dose should preferably be given at the same time to all participants every day.

The corresponding PLACEBO will be given in equal quantity, at the same time and respecting the same guidelines described above, to the participants who are allocated to the PLACEBO group.

According to the "Clinical study on the postprandial pharmacokinetics of AZVUDINE" (GQ-FNC-105, Henan Genuine Biotechnology Co, Ltd.), conducted in Beijing You'an Hospital affiliated with Capital Medical University under the responsibility of Wang Meixia in the period from Dec-2015 to May-2018, which is one of the reference of studies related to the preliminary stages of clinical development of AZVUDINE (FNC), the incidence of adverse effects observed in both regimens of experimental drug administration (fasting X postprandial) were similar to each other. All AEs were grade 1 in intensity, requiring no therapeutic management measures, thus demonstrating that the FNC administration regimen does not compromise the safety and tolerability of the investigational drug in healthy individuals.

In preliminary studies, dizziness (incidence ≥5%) seems to be a likely NCF-related AE. Although the last report of the Hope 4 study, provided by China, showed only one case of dizziness that was not related to the medication, the evening schedule for the administration of the experimental drug is a safety measure for participants in moderate stage with indication for hospitalization, because there is already weakness, where dizziness and fatigue are already symptoms of the disease, which in itself can increase the risk of falling during hospitalization. In this case, the administration schedule of the medication at 8 pm (after dinner and at bedtime) aims to prevent problems, taking into account the reported incidence of ≥5%. In the mild case, the participant is not debilitated and can have a variable schedule, preferably maintaining

the nighttime administration, as a safety measure, since it is an experimental drug, respecting the interval between doses to standardize the time for RT-PCR sample collection. However, there are no restrictions regarding meals.

The total course should not exceed 14 calendar days, even if a dose has been missed. The lost investigational product, if it occurs, shall follow the principle of "whether the missed medication schedule exceeds 1/2 medication interval or not." If the missed medication schedule is within the first 12 hours of the interval between two medications, the missed investigational product may be re-administered normally and the next administration may be performed according to the original schedule. If the time of the missed medication is more than the first 12 hours of the two-medication interval, the missed investigational product should not be re-administered and the next administration will be advanced.

If a participant is no longer under outpatient care, administration of AZVUDINE will be discontinued.

DOSE CHANGES

There are no clinical safety or pharmacokinetic data available for AZVUDINE in patients with renal and/or hepatic impairment. Given the benefit-risk ratio in patients with COVID-19, these participants are excluded from the study.

If the glomerular filtration rate (eGFR) decreases to <25mL/min, administration of the drug should not be given that day. The investigational product can be resumed the next day if the eGFR returns to ≥30 mL/min. If the participant's renal function worsens to the point that hemodialysis or hemofiltration is required, administration of the drug should be discontinued.

If ALT and/or AST increase to >5 times above normal, the dose of AZVUDINE should be suspended and not restarted until ALT and AST are ≤5 times the upper limits of normal.

6.1.5. **Overdosage**

AZVUDINE has undergone preclinical studies to identify adverse events related to long-term use of the medicine before it is used in patients with HIV (first use of AZVUDINE). In these preclinical tests, long-term exposure to the drug demonstrated that observable adverse events appear when the dose is exceeded by 20-30 times, but because the tests were done for much longer than suggested, AZVUDINE was shown to be safe at doses of 5mg/day, with the maximum dose over 14 days being 70mg in total.

Animal / human equivalent dose	Animal / Administration time	Total dose used in pre clinical trials over time
3,72mg	1 month	116,6mg
5,60mg	3 months	504,0mg
3,36mg	6,5 months (26 weeks)	655,2mg
3,72mg	9,75 months (39 weeks)	1.088,1mg

Table7: Relationship of dose and administration time

DRUG MANAGEMENT AND RECOVERY

The Principal Investigator, or someone delegated by him/her, must check the following items each time he/she receives the study drugs (experimental drug and PLACEBO): drug name; quantity; package specification; drug lot number; whether it complies with the quality inspection report; expiration date; whether the conditions during transportation comply with the storage conditions; check whether the outer packaging of the drug is in good condition; whether the package label is clear; check whether the drug labels are well attached; etc.

If storage conditions do not meet the specified requirements (airtight at room temperature) during transport, the batch of trial drugs should be stored under the original storage conditions, segregated from other stored products and reported to the

Principal Investigator, who, in conjunction with the sponsor, should judge whether the received batch may be used.

After completing the transfer sheet, it should be properly saved and filed in the post-judgment folder.

The Principal Investigator, or someone delegated by him/her, must keep accurate and complete records, including the receipt, accounting, dispensing, administration, retrieval, return, or loss of the investigational drug. The Principal Investigator must ensure that all investigational drugs are used only by the participants in this study, and that dosage and use must be in accordance with the research protocol.

Unused investigational drugs must be returned to the sponsor or disposed of by incineration, as per SMS Ordinance No. 028, of October 9, 2014, by means of the Drug Destruction Form, and must not be transferred to any other research participant.

Regardless of the form of disposal, the documentation generated by the chosen process should be archived along with the documents generated during the research so that they can be audited.

Study medications should be checked on a regular basis. In case of non-compliance events (wrong code, loss, lost drugs, damage, signs of loss of quality, etc.), the event should be recorded and reported immediately to the Principal Investigator and the sponsor.

DRUG INTERACTIONS

Throughout the trial period, the Principal Investigator and his team shall record information on the associated diseases and any therapeutic interventions (including drug therapy), surgical procedures etc. relating to the research participants. If possible, it should also include the diagnosis and date of onset of all diseases and the

date of symptom relief, as well as the name of the drug involved, the date of taking the drug and the description of the operation, etc.

CONCOMITANT THERAPIES AND PROHIBITED MEDICATIONS

No clinical drug interaction studies have been conducted between AZVUDINE and other experimental anti-SARS-CoV agents. However, in the exploratory studies conducted in China, patients were treated with experimental anti-SARS-CoV drugs such as arbidol, lopinavir/ritonavir, interferon-□ , hydroxychloroquine, osetalmivir, ribavirin, and others, and no AEs were attributed to drug interactions. Clinical trials with HIV-positive patients (GQ-FNC-103 and GC-FNC-104) showed that multiple doses of EFV and TDF significantly increased the degree of FNC exposure in the body. However, the sample size was small and not clinically relevant, so no dose adjustment was needed for FNC combined with EFV or TDF. In addition, another clinical trial (GQ-FNC-201) showed that there were no drug-drug interactions with marketed antivirals for HIV treatment (TDF and EFV), and that the therapeutic effect was comparable to the currently recommendedfirst-line treatment regimen: 3TC + TDF + EFV. All AEs reported in these trials were grade 1 and no additional treatment was required.

The use of some concomitant therapies may interfere with the efficacy, safety, and clarity of the data obtained from the research, but there is no evidence that administering AZVUDINE simultaneously with other agents may have no effect or adverse effects. Even so, the collection of this information will be added to the study data, including the use of vaccines for covid19.

At the beginning of the preparation of this protocol, vaccine development was not yet consolidated and the design of Azvudine clinical development protocols do not foresee the exclusion of volunteers who used vaccines. However, there was a scenario of accelerated development, approval, availability and distribution of new vaccines against COVID-19, as well as, reports of re-infection and non-immunization on time. On the other hand, information on drug interactions involving the new vaccines and other

therapeutic agents is scarce, except those involving the concomitant use of immunosuppressive agents, such as systemic corticosteroids. In this case, vaccinated individuals who have contracted the SARS-CoV-2 virus after vaccination and who are experiencing mild symptoms and meet the inclusion and exclusion criteria may participate in this study, since it is possible to monitor the viral load and antiviral action of Azvudine.

In the case of vaccination, after participation in the study, the 60-day follow-up period should be respected to verify the safety of the experimental drug. Although the mean lifetime of AZVUDINE at a dose of 5mg/day is 13.8 hours and the renal elimination is 24 hours (whole drug and metabolites), we must remember that the individual is recovering from an infectious condition.

The Responsible Investigator should also review medications prescribed for pre- existing comorbidities and evaluate whether these agents may lead to antagonism orsynergism with AZVUDINE or jeopardize participant safety, and should modify safety monitoring at his/her discretion if deemed necessary.

Use of another antiviral (concurrent infection, e.g. oseltamivir for an influenza virus, lopinavir/ritonavir for HIV, etc.) or immunosuppressive drugs for other medical conditions (tocilizumab for rheumatoid arthritis, hydroxychloroquine for lupus, etc.), or experimental drugs for COVID-19 in this study is prohibited. Individuals on alternative treatment should initiate treatment with AZVUDINE after the washout period described for each medication:

MEDICINE	***WASHOUT***
Oseltamivir	>3 days
Lopinavir	7 days
Ritonavir	7 days
Tocilizumab	6 weeks
Hydroxychloroquine	14 days

Table8: Drug washout period

Collection of information on concomitant medications is planned for visits 1-17. Concomitant medications should be reported in the appropriate eCRF. Report all prescribed medications taken during the study period.

Final guidance on AZVUDINE and potential drug interactions, if available, will be updated in the Investigator's Brochure and in protocol amendments.

PARTICIPANT MEMBERSHIP

A medication adherence is defined as the ratio of the number of actual medications taken to the number of medications that should have been taken during the dose administration period, multiplied by 100. Investigators should record the specific reasons for participants' inadequate adherence in the original medical records and in the eCRF for each visit. The definition of experimental medication adherence failure is: the proportion is less than 80% or greater than a 120%.

$$\text{Adherence assessment (\%)} = \frac{\text{actual amount of drug used}}{\text{intended amount of drug used}} \times 100$$

7. EVALUATIONS AND VISIT SHEDULE

Participants will be preferably assessed by the same evaluator at the beginning of the study (screening and randomization: D-1 and D0, respectively).

Follow-up includes assessment of participants who are not discharged after the end of treatment (D21) and two visits after outpatient discharge: two weeks (D28) and sixty days (D60).

Table 9shows the tests to be performed to assess eligibility criteria and monitor safety and efficacy during the study period.

The coronavirus viral load will be detected every 2 days. The treatment will have a maximum duration of 14 days, and may be interrupted early in case of obtaining two consecutive negative RT-PCR tests, or if the participant decides not to continue in the study.

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During the course of treatment, if the results of safety and efficacy testing show changes of concern, the investigators should determine whether or not the participant should continue with treatment. **The responsible investigator may adjust the procedures according to the research participant's condition and the results of clinical examinations.** The schedule of study procedures and examinations is detailed in Tabela 9, but not limited to the table.

Item	Requirements and outcome measures
Demographic information	Nationality, age, sex, height and weight
Anamnesis	Past/current medical history (history of illness, history of operation), history of anaphylaxis, medication use, alcohol use, drug abuse, participation in clinical trials, surgical treatment, or pregnancy/lactation.
Vital signs	Body temperature, heart rate, respiration, blood pressure
Digital Oximetry	-
Blood glucose	Blood glucose level
Detection of viral load by RT-PCR	Sampling of respiratory tract epithelium
ECG	12-lead or 18-lead ECG
CT lung	-
CBC	RBC, Hb, HCT, MCV, MCH, MCHC, WBC, NEUT #, LYMPH #, MONO #, EOS #, BASO #, PLT
Biochemical Examinations	AST, ALP, Cholinesterases, ALT, Alb, TP, TBIL, DBIL, GGT, Creatinine, UREA, UA, KP+P, NaP+P, ClP- P, Lactate, CaP+P, P.
Erythrocyte sedimentation rate (ESR)	ESR
Coagulation test	TP, TTPA, FIB, TT
Immunological markers	IgA, IgG, IgM, complement C3 and C4

Item	Requirements and outcome measures
Pregnancy test	Applicable to female participants

Table9: Parameters for evaluation of safety and efficacy

ASSESSMENT OF EFFECTIVENESS

7.1.1. Evaluation of primary efficacy

PRIMARY ENDPOINT:

- Proportion of patients hospitalized during the study until day 28, according to the WHO ordinal scale of clinical progression (Jun/2020), Score 4 to 10. [Period: until D15, D21 and D28, approximately 14-30 days.]

7.1.2. Secondary efficacy evaluation

SECONDARY OUTCOME:

- Proportion of participants with clinical outcome of **CURE** during the study; [Period: until D15, D21 and D28, approximately 14-30 days].
    - The clinical endpoint of cure is defined in this protocol as the absence of viral RNA in collected samples and clinical conditions for outpatient discharge[18].
- Improvement in clinical status in at least one category compared to screening on the Ordinal Scale of Clinical Improvement (WHO, Jun/2020) [Period: until D15, approximately 14 days].

[18] According to the WHO Working Group on Characterization and Clinical Management of COVID-19 Infection (July, 2020), clinical and virological absence of infection (non-detectable RT-PCR) is suggestive of CURE for initially infected participants.

- Duration of symptoms: time to normalization of body temperature, diarrhea, myalgia, fatigue, dizziness, cough and headache; [Period: until D15, D21 and D28, approximately 14-30 days].
- Changes in liver and renal function baseline; [Period: until D15, D21 and D28, approximately 14-30 days]
    - Laboratory tests performed on days D1, D3, D5, D7, D9, D13, D15, D21, D28 and D60
- Evaluation of negative conversion time of SARS-CoV-2 viral load by RT-PCR between AZVUDINE (FNC) group and control group; [Period: until D15, D21 and D28, approximately 14-30 days].
- Evaluation of the number of cycles for the detection of viral load of SARS-CoV-2 by RT-PCR and application of the standard curve for calculation of viral load; [Period: until D15, D21 and D28, approximately 14-30 days].
- Analysis of the relationship between the calculated and/or quantified viral load and the clinical evolution of the participants in the AZVUDINE (FNC) group compared to the control group; [Period: until D15, D21 and D28, approximately 14-30 days].
- Occurrence of drug interactions; [Period: until D15, D21 and D28, approximately 14- 30 days].
- All-cause mortality rate during the study; [Period: until D15, D21, D28 and D60, approximately 14-60 days].
- Frequency and intensity of adverse events, unexpected adverse events, and serious adverse events; [Period: until D15, D21, and D28, approximately 14-30 days]
- To evaluate the tolerability of AZVUDINE (FNC) use at 5mg/day regimen for up to 14 days: [Period: until D15, approximately 14 days].
    - Calculating treatment adherence;
    - Total time of use of AZVUDINE (FNC);

- Proportion of participants who withdrew consent;
- Proportion of participants who abandoned treatment.

SAFETY ASSESSMENT

Any clinical manifestations, severity, time of occurrence, duration, treatment methods and prognosis shall be recorded in the participant's eCRF. The following safety parameters shall be assessed:

- Frequency and intensity of adverse events;
- Frequency and intensity of unexpected adverse events;
- Frequency and intensity of serious adverse events;
- Adverse events related to the experimental drug;
- Abnormal laboratory test values;
  - Routine blood work (WBC count, RBC count, Hemoglobin, Platelet count, Neutrophil count, Lymphocyte count), ESR, IL-6, C-reactive protein, Liver function (AST/TGO, ALT/TGP, GGT, BT, ALP/FA), renal function (Glomerular filtration rate, serum creatinine), coagulation function (APTT, TAP/PT, TT, FIB), blood lactate, cardiac enzymes and markers, procalcitonin, T lymphocyte subsets;
- Vital signs (body temperature, heart rate, respiration, blood pressure);
- Pregnancy testing of women of childbearing age;
- EKG.

Clinical safety shall be assessed by means of spontaneous participant reports or direct observation of the investigator or his/her team on a daily basis, or by asking participants about adverse events on a non-induced basis. Participants will be screened with blood tests, imaging tests, respiratory tract tests, and vital signs as listed Table 9, as indicated, but not limited to, these items.

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VISIT SCHEDULE

The research site is responsible for defining the strategies for recruitment and initial screening of research participants, knowing that the screening process may be suspended at any time if exclusions are identified by the study team.

VISIT 1: INITIAL (D-1)

Patients with mild symptom for COVID-19, with indication for home treatment, aged above 18 years, will be invited to participate in the study. **If the volunteer accepts to be a participant**, he/she and his/her companion, if applicable, will be informed of the study procedures, including the laboratory exams of the screening period, the criteria for inclusion and exclusion from the study, as well as their rights and duties. Only after signing the TCLE, the patient will be included in the study and the eligibility criteria can be explored after the anamnesis, performance of laboratory tests, collection of nasopharyngeal and oropharyngeal swab samples for RT-PCR testing and imaging tests. **Confirmation of study participant eligibility will be assessed once all screening data are available.**

The following procedures will be performed at visit V1:

- Invitation to volunteer;
- Consent process and signing of the TCLE;
- Verification of inclusion and exclusion criteria;
- Demographic data collection;
- Anamnesis;
- Collection of clinical history data;
- Vital signs monitoring;
- Collection of blood samples and respiratory specimen(nasal and oropharyngeal swabs) for screening tests;
- Performing pregnancy test (women of childbearing age).
- Eligibility assessment and confirmation with Principal Investigator.

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VISIT 2: RANDOMIZATION (D0)

After confirmation of eligibility for the study by meeting the inclusion and exclusion criteria and performing the screening procedures, the eligible individuals will be directed to Santa Casa de Misericórdia de Campos for randomization (experimental or control group), performance of complementary exams, outpatient care and information about their home follow-up[19], starting to follow the Treatment Protocol for COVID-19 - 2nd Version (July 02, 2020) of Santa Casa de Misericórdia de Campos.2starting to follow the Treatment Protocol for COVID-19 - 2nd Version (July 02, 2020) of Santa Casa de Misericórdia de Campos.

The following procedures will be performed at visit V2:

- Validation of eligibility by the Principal Investigator;
- Directing the participant to Santa Casa de Misericórdia de Campos;
- Randomization of research participants;
- Performance of complementary exams, outpatient care and information on home monitoring;
- Dispensing of the investigational drug;
- Accounting for the investigational drug.

VISITS 3-16: PROCEDURE (D1-D14)

The treatment period will start at D1 according to randomization.

The treatment will last up to 14 days and may be stopped early in case of treatment success (2 consecutive negative RT-PCR tests), participant dropout, or at the discretion of the Principal Investigator, if deemed necessary for the safety of the participant.

[19]Manejo clínico do coronavírus (covid-19) na atenção primária à saúde, versão 7, abril 2020, Secretaria de Atenção Primária à Saúde (SAPS), Ministério da saúde.

The experimental drug should be administered daily, with a variable schedule, but preferably maintaining nighttime administration, as a safety measure, since it is an experimental drug, and respecting the interval between doses in order to standardize the time for RT-PCR sample collection. However, with no restrictions on meals.

For **Early Termination (ETA)** of treatment, participants should follow the routine for visit 17: End of Treatment (D15), in advance.

The Principal Investigator for the study will be available to answer any questions.

The following procedures will be performed:

Home visits for the supply of the experimental/placebo drug, every 48 hours, coinciding with the RT-PCR collections. Every 48hs the participant will be provided with the dose of the day and the next day, when the nurse goes to the residence to collect blood and nasal/oropharyngeal swab sample for RT-PCR of the home monitoring schedule. There is also the concern of not giving a bottle with 35 tablets of AZVUDINE 1mg to the participant, as he/she may share with family members.

- The daily administration of AZVUDINE or PLACEBO will be oriented to follow the same schedule, but preferably maintaining the night administration, as a safety measure, since it is an experimental drug and, respecting the interval between the administrations for the standardization of the time for the collection of RT-PCR samples. However, with no restrictions on meals;
- Monitoring by telephone, web technology and telemedicine[20,21], daily;

[20]Ordinance No. 467 of March 20, 2020, published on an exceptional and temporary basis, on Telemedicine actions, in order to regulate and operationalize the measures to address the public health emergency of international importance arising from the epidemic of COVID-19.
This ordinance authorizes, on an exceptional and temporary basis, Telemedicine actions of interaction at a distance. They can include pre-clinical care, care support, consultations, monitoring and diagnosis, performed through information and communication technology, within the scope of SUS.
[21] Clinical management of coronavirus (covid-19) in primary health care, version 7, April 2020, Secretariat of Primary Health Care (SAPS), Ministry of Health.

- According to the recommendations of the Ministry of Health for clinical monitoring, patients with influenza syndrome in outpatient monitoring should remain in isolation for 14 days from the date of onset of symptoms. The monitoring should be done every 24 hours in people over 60 years and carriers of comorbidities at risk and every 48 hours in others, preferably by phone. If necessary, face-to-face care should be provided, ideally at home. All household members should be considered as contactors and should also be kept away for 14 days and followed up, besides being stratified appropriately in case they start showing symptoms. If there is worsening of the patient in home treatment or the development of severe symptoms in relatives of the patient, referral to other levels of care of SUS[22] becomes mandatory.

- Attendance in person of the participant in case of drug interactions and AEs;
- Monitoring of symptoms, vital signs, oximetry and blood glucose every 48h during home visits;
- Collection of blood samples and respiratory sample (nasal and oropharyngeal swab) for safety and efficacy monitoring tests every 48 hours during home visits;
- Examination of CT scan of the lung and electrocardiogram on days D1 and D15;

The Principal Investigator may adjust the procedures performed during treatment according to the condition of the research participant and the results of clinical examinations.

VISIT 17: END OF TREATMENT (D15)

[22]Clinical management of coronavirus (covid-19) in primary health care, version 7, April 2020, Secretariat of Primary Health Care (SAPS), Ministry of Health.

At the end of treatment or discontinuation of the investigational drug or PLACEBO, all participants should undergo procedures and tests for safety and efficacy monitoring. The procedures should be performed prior to discharge from the hospital approximately 24 hours after the last dose of the investigational medication or PLACEBO, because for the 5 mg dose of AZVUDINE, the mean half-life is 13.8 hours and is excreted in the urine within 24 hours.

The treatment will last up to 14 days and may be stopped early in case of treatment success (2 consecutive negative RT-PCR tests), participant dropout, or at the discretion of the Principal Investigator, if deemed necessary for the safety of the participant.

The following procedures will be performed at visit V17:

- Outpatient care of the participant, at Santa Casa de Misericórdia de Campos;
- Monitoring of drug interactions and AEs;
- Monitoring of symptoms, vital signs, oxygen and blood glucose measurement;
- Collection of blood samples and respiratory sample (nasal and oropharyngeal
- swab) for safety and efficacy monitoring tests;
- CT scan of the lung and electrocardiogram.
- Performing pregnancy test (women of childbearing age).

VISIT 18: MONITORING (D21)

Participants who, even **after completion of treatment, escalate to a hospital admission must undergo the procedures and tests for safety and efficacy monitoring** expected to occur within approximately **7 days after administration of the last dose of** the investigational drug or PLACEBO.

The following procedures will be performed at visit V18:

- Monitoring of drug interactions and AEs;
- Monitoring of symptoms, vital signs, oxygen and blood glucose measurement;

- Collection of blood samples and respiratory sample (nasal and oropharyngeal
- swab) for safety and efficacy monitoring tests;
- CT scan of the lung and electrocardiogram;

Participants who are not discharged from outpatient clinics after the end of treatment should follow the D28 and D60 follow-up schedule. However, safety procedures and examinations will not be exclusive to these days and research participants who aggravate will be assisted at all times by the Principal Investigator and his/her team.

VISIT 19: MONITORING (D28)

Two weeks after outpatient **discharge,** the volunteers should return to the research center for procedures and tests to monitor safety and efficacy after the end of treatment.

The following procedures will be performed at visit V19:

- Monitoring of symptoms, vital signs, oxygen and blood glucose measurement;
- CT scan of the lung and electrocardiogram.

The Principal Investigator may adjust the procedures performed during the outpatient visit according to the research participant's condition and the results of clinical examinations.

VISIT 20: MONITORING (D60)

60 days after outpatient discharge the volunteers should return to the research center to undergo procedures and exams for safety and efficacy monitoring.

The following procedures will be performed at visit V20:

- Monitoring of symptoms, vital signs, oxygen and blood glucose measurement.
- Collection of blood for safety and efficacy monitoring tests;
- Performing pregnancy test (women of childbearing age).

The Principal Investigator may order tests according to the participants' previous results.

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Schedule of visits and procedures

WEEK/DAY	D-1	D0	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D8	D9	D1 0	D1 1	D1 2	D1 3	D14	D15 AFTER THE START OF TREATMENT	D21[5]AFTER THE START OF TREATMENT	D28AFTER THE START OF TREATMENT	D60 AFTER THE START OF TREATMENT[3]
VISIT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
VISIT BREAK	± 4 days	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Before outpatient discharge	± 3 days	± 3 days	± 3 days

PHASES	SCREENING	RANDOMIZATION	BLIND CHARACTER TREATMENT[1]														END OF TREATMENT[2]	FOLLOW-UP		
ICF	X																			
DEMOGRAPHIC INFORMATION	X																			
ANAMNESIS	X																			
ELIGIBILITY CRITERIA	X																			
RANDOMIZATION		X																		
DISPENSING THE MEDICATION		X																		
DRUG ACCOUNTING		X																		
SYMPTOM MONITORING	X		X		X		X		X		X		X		X		X	X	X	X
VITAL SIGNS	X		X		X		X		X		X		X		X		X	X	X	X
FINGER OXYGEN PULSE	X		X		X		X		X		X		X		X		X	X	X	X
BLOOD GLUCOSE			X		X		X		X		X		X		X		X	X	X	X
PREGNANCY EXAM[4]	X																X			X

RT-PCR	X		X		X		X		X		X		X		X		X		
BIOCHEMICAL EXAMS	X		X		X		X								X		X		X
CT			X												X		X		
ECG			X												X		X		
BLOOD COUNT			X		X		X								X		X		X
FUNCTION OF COAGULATION			X		X		X								X		X		
ESR/VHS			X												X		X		
PHOSPHORUS IN URINE			X												X		X		
AZVUDINE OR PLACEBO ADMINISTRATION			X																
MONITORING OF DRUG INTERACTIONS			X												X				
AE MONITORING			X												X				

Table10: Visit schedule and study procedures.

[1]Day 1 of administration will be recorded as the first day (D1) and the days of treatment continue until day 14 (D14). The Principal Investigator can adjust the procedures performed during treatment according to the condition of the research participant and the results of clinical examinations (the condition manifests more quickly in some patients and these patients should be closely observed).

[2]The examinations and procedures provided for in visit 17 (D15) must be carried out by all participants as soon as the treatment ends, including those who interrupted treatment early (ET – Early Termination) and for any other reason.

[3].*The procedures and exams scheduled to be performed at the D28 and D60 visit will be determined by the Principal Investigator, according to the participants' previous exams.*

[4]*For women of childbearing age only.*

[5]*The procedures of visit 18 (D21) will only be carried out on research participants who are still hospitalized.*

ADDITIONAL VISITS

In addition to the visits provided herein, **extra visits may be made to** perform procedures indicated by the Principal Investigator prioritizing the safety of the visiting participant.

VIEWING WINDOW

To be eligible to participate in the study, among the proposed inclusion criteria is the presence of a positive diagnostic test for SARS-CoV-2 by molecular amplification of the virus in a respiratory sample (nasopharyngeal, oropharyngeal, lower respiratory tract, e.g.: sputum) collected <96 hours before randomization. For this reason, the time between screening and randomization should not exceed 4 daysU.

To attend the end of follow-up visits **(D28 and D60)** participants will have the possibility to **advance or delay by up to three days** from the initially scheduled date.

8. ADVERSE EVENT REPORTING

Adverse event (AE), according to ANVISA, means "any adverse medical occurrence in a patient or clinical trial participant to whom a pharmaceutical product was administered and that does not necessarily have a causal relationship to the treatment. As a result, an AE can be any unfavorable and unintended sign, symptom, or disease (including results outside the reference range), associated with the use of a product under investigation, whether related to it or not". The adverse event may be related to the following situations:

- Exacerbation of pre-existing medical conditions and/or the original disease (including symptoms, signs, laboratory abnormalities);
- Any new adverse events or new adverse medical conditions (including symptoms, signs, newly diagnosed diseases);
- Abnormal values of clinically significant laboratory or imaging tests or results that are not caused by concomitant diseases.

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SERIOUS ADVERSE EVENT(SAE)

According to ANVISA's definition, serious adverse event is "that which results in any experience with medication [...], occurring at any dose and resulting in any of the following outcomes":

- Death;
- Imminent threat to life;
- Persistent or significant disability/disability;
- Inpatient hospitalization or prolonged hospitalization;
- Congenital anomaly or birth defect;
- Any suspected transmission of an infectious agent via a medicinal product;
- Other clinically significant events: events that may lead to one of the above, including but not limited to the following events, such as allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood system cachexia or seizures not requiring hospitalization, potential drug-induced liver injury, spread of pathogens (non-pathogenic or pathogenic) via the study medication, pregnancy, overdose, secondary tumors, etc, are generally considered serious, although they may not lead to death or hospitalisation.

The Principal Investigator shall provide the criteria for the severity of the SAE, based on the study protocol and his/her own medical judgment, the eCRF and the results of the assessment of the expected occurrence of the SAE and the correlation (cause and effect) with the study medicinal product.

If the causality assessment performed by the Principal Investigator is missing or cannot be obtained, the sponsor will make the judgment and provide the final assessment, which will subsequently be confirmed by the Principal Investigator. If the Principal Investigator cannot determine whether or not the AE belongs to an SAE, then it will be considered an SAE until its nature is proven.

When any SAE occurs in the study, whether related to the investigational drug or not, the investigators should provide immediate assistance.

Planned or elective hospital admissions for administrative reasons (such as annual physical examination) or prolongation of length of stay unrelated to worsening AEs will not be considered SAEs. **The following situations will not be reported as SAE:**

- Treatments/procedures for a pre-existing condition/disease reported in the screening period;
- Hospital readmission due to the original disease with no new AE and no exacerbation of the original disease (such as verification of laboratory abnormalities that persisted until now);
- Invasive (such as surgery) and non-invasive diagnostic or therapeutic procedures should not be reported as an AE. However, it should be reported when the condition leading to that operation meets the definition of AE. For example, acute appendicitis that occurred during the AE monitoring period should be reported as AE and therefore an appendectomy should be recorded as the treatment method for this AE.
- AEs leading to hospitalization or prolonged hospitalization in clinical trials should be considered as AEs. The following situations of hospitalization are not considered as AEs: rehabilitation institutions; nursing home; routine emergency admission; same-day surgery (as outpatient/same-day/outpatient surgery).
- **Hospitalisation due to symptoms and signs of disease progression during the trial should not be reported as an SAE**, while death due to disease progression (including signs and symptoms of disease progression) should be reported as an SAE. The events causing death should be reported as an SAE, for example, if cancer leads to the participant's death during the trial.

SUSPECTED UNEXPECTED SERIOUS ADVERSE EVENT (SUSAR)

Unexpected serious adverse reaction whose nature, severity, consequence or frequency is not consistent with the expected risks described by the information in the most current investigator's brochure. All unexpected serious adverse reactions occurring during the clinical trial which are definitely related to or suspected of the investigational product are SUSAR. The assessment of SUSAR should be performed considering the following aspects:

- If the AEs are listed in the investigator's brochure (BI);
- Although the AEs are listed in the BI, whether or not their severity is consistent with what is described in the BI;
- Although the adverse events are listed in the BI, the frequency of occurrence is higher than that described in the BI;
- Other situations.

ADVERSE EVENT OF SPECIAL INTEREST

According to the results of current clinical trials, adverse events that may be related to the product are:

- Dizziness;
- Headache;
- Decreased phosphorus in the blood;
- Increased alanine aminotransferase;
- Increased aspartate aminotransferase;
- Increased total bilirubin;
- Increased γ-glutamyl transferase;
- Decreased neutrophils;
- Increased glucose in the blood.

However, according to the AE incidence data reported in the clinical development phases of the FNC performed previously, among the AEs already described, AEs of special interest will be considered:

- Changes in markers of liver functionP18F[23]P:
  - Elevation of AST/TGO above reference values
  - Elevation of ALT/GTP above reference values
- Changes in markers of renal function:
  - Diminuição de Taxa de Filtração Glomerular estimada (eGFR) <30mL/min
- AE grade 3 related to dizziness, due to increased risk of falling.

The Principal Investigator shall classify the degree of intensity and the correlation (cause and effect) between the test drug and the AE of special interest, based on the study protocol and on his own medical judgement, recording in the specific eCRF. The sponsor should be notified within 7 days of the occurrence of AE of Special Interest.

AES MONITORING

If there is any abnormality or AE, severe or not, unexpected or not, during or after treatment, follow-up should be made until clear outcome of recovery or stable status. Individuals with AEs developed after discharge should contact the Principal Investigator or his/her team to determine what action should be taken for that individual to fully recover from the AE.

After the end of the trial, if participants have AEs again, which are or may be related to the use of the investigational drug, the PI should inform the sponsor in time.

For all SAEs (including those that are still in the developmental stage after the end of the trial and until the end of follow-up of the last patient), the Principal Investigator shall follow the patient until recovery, stabilization, or death to ensure that all problems are resolved. Until the EAG has been resolved, the Principal

[23] TGO Adulto 12 a 46 U/L; TGP Adulto 03 a 50 U/L (Fonte: Instituto Hermes Pardini)

Investigator should keep the sponsor informed. Detailed follow-up information should be provided,e.g. whether or not special treatment is needed after the trial, whether or not hospitalisation is required, etc

AE INTENSITY

The intensity of AE in this study is classified according to the World Health Organization criteria, adopted by ANVISA:

- **Mild:** present less than 25% of the time, with an intensity that a person can tolerate and has rarely happened since the start of treatment.
- **Moderate:** present less than 50% of the time, with an intensity that interferes with the individual's day-to-day activities and has occurred occasionally since the beginning of treatment.
- **Severe:** present more than 50% of the time, with an intensity that partially alters the individual's daily life and has happened frequently since the beginning of treatment.
- **Complete impairment:** present more than 95% of the time, with an intensity that completely alters the individual's daily life and has occurred every day since the beginning of treatment.
- **Not specified:** not enough information to specify the intensity.
- **Not applicable:** it is inappropriate to use a gradation (e.g. menstrual functions).

CAUSALITY BETWEEN AES AND STUDY DRUGS

All AEs, serious or not, unexpected or not, should be reviewed by the Principal Investigator for relationship analysis with the study drug, according totable11. The following factors should be taken into consideration when analyzing the correlation between AEs and the study drug:

- Whether there is a reasonable time sequence between the time of drug action and AEs, and it was possible to observe a plausible relationship between the drug administration and the event that occurred;
- Whether AEs can be explained by the original disease, by another intervention, or by environmental factors;
- whether there has been a response, pharmacological or pathological, after discontinuation or reduction of test drug dose;
- If the same AE reappeared after resumption of the suspected drug, whether re-exposure of the participant to the study drug was possible;
- Whether the clinical or pathological manifestation of the AE is consistent with knowledge of the pharmacological and toxicological classification of the known test drug;
- Whether the data on the EA is clear, complete and traceable.

CORRELATION	CRITERIA
Sure/Defined	• There is a reasonable time sequence between the AE and the medication; • AE cannot be explained by other factors; • There was a response after dose withdrawal or reduction; • The same AE reappeared after resumption of the drug, if re-exposure was possible; • The AE is consistent with the medication.
Probable	• There is a reasonable time sequence between the AE and the medication; • Unlikely that the AE is attributable to other factors; • There was a response after dose withdrawal or reduction; • Re-exposure of the drug was not possible or there is

CORRELATION	CRITERIA
	no clear information.
Possible	• There is a reasonable time sequence between the AE and the medication; • AE can be attributed to other factors as well; • There is no clear information on the response after dose withdrawal or reduction
Unlikely	• There is no clear sequence of reasonable time between EA and medication; • EA can be attributed other factors as well;
Conditional/Unclassified	• There is no time sequence between the AE and the medication; • The data is unclear for; • Additional data is under investigation
Inaccessible/Unclassifiable	• The narrative of the account suggests an AE; • The information is insufficient or contradictory; • The data cannot be supplemented or verified.

Table11: Causality relationship between AEs and study drugs

AE COLLECTION AND REGISTRATION

Any information relevant to the study should be recorded from first administration to the end of follow-up. AEs will be monitored throughout the study and the Principal Investigator, or someone delegated by him/her, will be responsible for recording all adverse medical events observed during the study, in detail, in the source documents and in each participant's eCRF during the study period from the first dose to the end of follow-up, irrespective of severity and causal relationship to the study drug.

All EAGs must also be recorded in eCRF as soon as they occur, and the information provided in the EAG report form must be consistent with the data related

to the event that is recorded in eCRF. All AEs, SAEs and special interest AEs must include the following content in detail:

- **Description:** Events should be described in medical terms, rather than the words used to record participant reports in the original document. If applicable, symptoms, signs, laboratory abnormalities and diagnosis should be included. If the same AE occurs more than once in the same participant and the participant recovered from both events, the AE should be recorded twice.
- **Date of occurrence:** the date when the AE first occurred in the participant or the date when the symptoms related to the AE occurred shall be recorded. If the AE is the test result of an abnormal laboratory test or test of clinical significance, the date of occurrence is the date the test was performed.
- **Date of result:** the date of resolution of AE-related symptoms should be recorded.
- **AE intensity and severity:** classified as: (1) mild, 2 (moderate), 3 (severe) or 4 (complete/severe impairment).P19F[24]
- **Causal relationship between AE and study medication:** as perTable11.
- **Measures for the study drug:** it should be recorded whether the participant continued using the drug or had to stop using it as a result of the AE or SAG. If the reaction occurred after the end of treatment, this item does not apply.
- **Measures for AE:** It should be recorded what measures were taken to solve the AE, such as drug therapy and/or non-drug therapy, hospitalization, surgery, among others. If no action was taken, this fact should be reported.
- **Outcome:** the disappearance of signs or symptoms; recovery with sequelae; stability; worsening; death; or loss to follow-up should be recorded.

[24] Manual para notificação de eventos adversos e monitoramento de segurança em ensaios clínicos / Brasília. Anvisa, 2016

- **Significant AE:** refers to any obvious AE, hematologic, or other laboratory test abnormality that necessitates targeted clinical interventions (such as drug withdrawal, dose reduction, and/or routine treatment), except for SAE.

If an AE or SAE occurs during the trial, whether or not related to the study medicinal product, the investigators shall take appropriate treatment measures immediately to ensure the safety of the participants.

EVALUATION OF ABNORMALITIES OF CLINICAL LABORATORY TESTS AND OTHER ABNORMALITIES CONSIDERED AS AE

Laboratory test abnormalities of no clinical significance are not recorded as AE or SAE. Laboratory test abnormalities of clinical significance (flagged as "abnormalities of clinical significance", such as clinical blood routine, blood biochemistry, etc.) and other abnormalities (such as ECG, vital signs), that meet the definition of AE or EAG following the principal investigator's assessment and judgement, or that require individuals to receive specialised routine treatment, should be recorded as AE or EAG.

If laboratory abnormalities are part of the syndrome, record the syndrome or diagnostic findings (e.g., anemia) rather than the laboratory test results (i.e., decreased hemoglobin).

PREGNANCY

Women of childbearing age with suspected pregnancy or a desire to become pregnant should not take part in this study. However, women using contraception may participate in the study. The following methods will be accepted: subcutaneous implant, IUD, SIU, hormonal injection, pills, condoms, sterilization and vasectomy. A description of contraceptive methods is provided in Appendix 6. Pregnancy testing will be done PRIOR to the start of the research (during the recruitment phase), at discharge, 24 hours after the last use of AZVUDINE, when the drug is no longer in your body. If you are a man who decides to participate in the study, it is recommended that you avoid pregnancy for your partner as well.

- ✓ Remembering that the prescription of AZVUDINE will be made up to 14 days, and may be suspended before in case of discharge;
- ✓ Remembering that the treatment is relatively short, we suggest that there are precautions regarding pregnancy at this time;
- ✓ If you become pregnant, you will no longer receive AZVUDINE and the team will monitor you and your baby's health until after delivery for as long as the need exists.

Although doses 1000 times above the clinical dose do not cause genetic mutations by the Ames test, nor chromosomal aberrations in cells in vitro, this does not mean it is the right time to get pregnant, even because, your health is requiring hospital care."

If a female participant or the spouse of a male participant becomes pregnant during the trial, the event is recorded and reported to the regulatory and ethical authorities and the sponsor. If the pregnancy is discovered during the treatment period, the use of the trial drug should be discontinued and the pregnancy event should be reported in eCRF. If the pregnancy is discovered in the follow-up period, it should also be reported in the eCRF. If the pregnancy occurs within 6 months after the end of the trial, the pregnancy event should be reported to the sponsor and ethical and regulatory authorities. In all cases, mother and baby should be followed up until delivery. Pregnancy complications and selective termination of pregnancy for medical reasons should be reported as AE or EAG, and spontaneous abortion should be reported as EAG.

NOTICE OFAE

AEs, EAGs and SUSARs should be recorded in detail in source documents and in the participant's eCRF, according to the MedDRA terminology (version 23.0) to specify the event that occurred. Non-serious AEs should be reported to the regulatory

and ethical authorities in the follow-up reports of the investigational medicinal product.

The Principal Investigator shall notify the sponsor of the occurrence of AEs according to the ICH Guidelines for Good Clinical Practice GCP/ICH - ICH E6, as well as the RDC No. 09 of February 2015 (ANVISA).

The Principal Investigator shall notify the sponsor of the occurrence of EAG or SUSAR within 24 hours of knowledge of it, in a dated and signed document, regardless of the causal relationship with the study drug. Additional information should be reported as soon as available. The sponsor should report the occurrence of unexpected, possible, probable or definite EAGs to the ethical and regulatory authorities within 7 calendar daysP20F[25]P.

For EAG or SUSAR whose information is temporarily incomplete and uncertain, it shall also be reported in a timely manner (within 8 calendar days), and shall be reported in the form of a follow-up report to the regulatory authorities after further information is obtained.

All other unexpected serious adverse events whose causality is possible, probable or defined in relation to the products under investigation shall be reported to Anvisa within 15 (fifteen) calendar days from the knowledge of the case by the sponsor.

9. CONCLUSION AND DISCONTINUATION OF THE STUDY

Information about the completion or discontinuation of the trial research participant and the reason for study discontinuation will be recorded in the appropriate CRF.

[25] https://formsus.datasus.gov.br/site/formulario.php?id_aplicacao=3961

PARTICIPANT COMPLETION

At the end of the treatment period of up to 14 days or when the coronavirus viral load detection by RT-PCR is negative for two consecutive times, considering a difference of at least 24h between the two sample collections, the study medication will be discontinued and the participant follows with the standard treatment therapy.

Treatment may also be discontinued if the Principal Investigator thinks it is better for the participant's safety or if the participant decides not to continue with treatment. In either situation, follow-up will be done on days D28 and D60 after the end of treatment.

The participant will only be discharged from the hospital at the end of treatment after medical evaluation by the Principal Investigator. The participant will be required to return to the hospital for follow-up visits. After the last visit, the individual's participation in the study will be considered terminated.

The need for additional intervention or the occurrence of safety endpoints/endpoints do not constitute study completion and are not criteria for withdrawal of the study or study medication.

STUDY CONCLUSION

The study will be considered completed when the last participant completes their last visit.

INTERRUPTION OR DISCONTINUATION OF THE STUDY PARTICIPANT

Participants are free to withdraw consent and/or discontinue study participation at any time without prejudice to subsequent standard of care. The wishes of the research participant and their medical care should take precedence over any research intent or procedure associated with the study. The research participant's participation in the trial may also be discontinued at any time at the discretion of the investigator or sponsor. Participants may also be discontinued from the trial if the trial

is terminated. Thus, the following criteria define the discontinuation of a research participant:

1. Voluntary withdrawal of the participant at any time and without any damage to his/her health;
2. Refusal or inability to perform and/or complete the study procedures, when applicable;
3. Loss of contact with the participant for follow-up;
4. Death;
5. Closure of the study.

The participant who discontinues the study shall complete the safety and efficacy evaluations, despite discontinuation of study medication, scheduled for study termination (V17) listed in the schedule of procedures detailed in Table 10, as close as possible to the participant's last dose of study medication. Data from the visit, including the primary reason for premature discontinuation of the study, will be recorded in the study end electronic clinical record (eCRF).

If participants withdraw from the study due to subjective motivations, the Principal Investigator should make reasonable efforts to contact the participants, or other contacts provided by them, by phone or email to determine the reasons for complete withdrawal as accurately as possible. If possible, the Principal Investigator should describe the detailed reasons for withdrawal in the original record and in the eCRF.

DISCONTINUATION OR INTERRUPTION OF STUDY MEDICATION

If during the course of the study participants develop any more serious condition requiring intubation, considering that the drug is administered orally, the drug will be discontinued for various reasons:

- We don't know which arm the participant is in;

- Inability to standardize the protocol of conventional treatment and monitoring of each co-participating unit in the period of hospitalization.

If during the course of this study participants exhibit any of the following criteria below, the Principal Investigator shall judge whether they should discontinue study medication:

- Confirmed by retest, TBIL≥ 5 times of the upper limit of normal value, ALT and AST ≥5 times of the upper limit of normal value;
- If eGFR decreases to an eGFR <25 mL/min and the participant's renal function worsens to the point of requiring hemodialysis or hemofiltration;
- Any situation that, in the judgment of the Principal Investigator, puts the safety of the participant at risk.

Participants who discontinue study medication early should be encouraged to continue the post-treatment visit to continue collection of safety and efficacy data despite discontinuation of study medication. Participants who agree to continue regularly scheduled study visits should complete the study termination assessments (V17) listed in the schedule of procedures detailed in Table 10 as close to the participant's last dose of study medication as possible. The visit data, including the primary reason for discontinuation of study medication, will be recorded in the eCRF. Subsequent study visit data, if applicable, will be recorded in the visit-specific eCRF, and the participant's final study visit will be recorded in the study termination eCRF.

If the reason for participants' discontinuation is an AE, they will continue to receive rigorous medical care until the clear outcome of recovery from AE or steady state. See Section 7 of this protocol for detailed follow-up information. For women who became pregnant during the trial period, the Principal Investigator may refer to item 8.9of this protocol for treatment.

Participants discontinuing study medication who are unwilling to continue with regular study visits, but are willing to continue to provide their information for study

use will be encouraged to remain on the study. The investigator will contact the sponsor's representative to determine the best approach based on the participant's situation, even if physical and laboratory examinations are not performed, monitoring for drug interactions and adverse events may be considered for these cases.

LOSS OF MONITORING

The investigator should make every effort to contact participants who do not return for scheduled visits so that they are not declared as "lost to follow-up. Participants will be considered "lost to follow-up" only after reasonable documented attempts to contact the participant have failed. Such attempts include, but are not limited to, the following:

a. Contact all telephone numbers of the participant and their listed contacts (to be recorded in the source document when the participant enters the study), as applicable.
b. Send e-mail, SMS and or other form of electronic contact to the participant's addresses and contacts, as applicable.
c. Review available medical records/notes regarding details of hospitalizations, clinic visits, or other procedures that may indicate the participant's condition, as applicable.
d. Check local, regional, and national public records to locate the participant or seek his or her death certificate, as applicable and permitted by law.

The information and dates of attempted contact should be documented in the participant's records and the participant's final status should be recorded in the appropriate eCRF. Once these steps have been exhausted and documented, the sponsor or its representative should be contacted for further guidance, if applicable.

EARLY INTERRUPTION OR DISCONTINUATION OF THE STUDY

The study may be terminated by the sponsor, Principal Investigator, or at the request of local health and ethics authorities.

Among the causes for termination may be the detection of technical problems, the possibility that the security of the participant is not guaranteed, or even the possibility of natural disasters, civil wars, public calamities, widespread interruption of

power and internet supply, paralysis of postal services (strikes) or other cause that makes it impossible to conduct the survey.

Other possible reasons for interruption or suspension of the Principal Investigator or research center include, but are not limited to:

- Unsatisfactory participant inclusion rate or problems related to adherence to eligibility criteria;
- Serious breaches or repeated deviations from the clinical protocol;
- Submission of inaccurate, incomplete and/or out-of-period data;
- If any EAG or technical safety issues occur, i.e. the trial may be terminated when adverse event rates in the trial or other studies with the product indicate a possible hazard to the health of participants caused by the medicinal product;
- Persistent failure to adhere to the clinical protocol, Good Clinical Practice guidelines, regulations or the clinical trial contract;
- Suspension of the research center by the REC;
- Fraud or fraudulent conduct;
- Request from the Principal Investigator (e.g., inability to conduct the study).

In case of discontinuation of the study, for any reason, the participants shall be notified immediately by the Principal Investigator and shall have the continuity of their treatment, regardless of the study, being assured the right to immediate and free assistance until the outpatient discharge and the scheduled return visits.

10. DATA MANAGEMENT

DATA MANAGEMENT

Research-IGZ 2.0 software will be used for data management.

The key data management processes are listed below, and others are included in the Data Management Plan (DMP) in detail in ANNEX 2.

The DMP, as the data management guidance document, is written by the data manager (DM) and approved by the sponsor. Data management will be carried out according to the time, content and method defined by the DMP.

RESEARCH-IGZ 2.0 DATA MANAGEMENT

- **Electronic case report form (eCRF):** is designed is configured according to logic check and is released for use after passing the test and sponsor approval.
- **Data entry:** eCRF data come from source documents, which are obtained through the entry of participants' data by the team responsible for data entry, according to the eCRF filling explanation. Data are collected directly on a tablet with cloud storage, and each user has access to his/her profile according to his/her permissions;
- **Source documents:** Are scanned and attached, when necessary, according to the phase of the study, to facilitate data verification;
- **Verification of source data:** the monitor through its access permission can remotely monitor system data, access the attached source documents and validate the processes. Thus, it checks the consistency of the data globally, being able to intervene through questioning, on-site visits or checking routines;
- **Questions and answers about data:** questions come from the Research-IGZ logic check question system, the monitor, the DM etc. the Principal

Investigator, or someone delegated by him/her, should answer the questions in a timely manner. If necessary, the DM and monitor may question again until the data are 'cleared'.

- **Assinatura do investigador principal**: após a entrada dos dados e dos dados de origem, o Investigador Principal deve realizar a verificação e confirmação por login e senha. Ao final, a assinatura digital será requerida. Se houver alguma revisão de dados após a assinatura, eles devem ser assinados novamente.
- **Reporting:** Research-IGZ 2.0 can provide reports on adverse events and the progress of study participants, being identity blind but non-blind to the system, and can generate partial data for efficacy evaluation, AE, schedule, and anything optimized from the database, without breaking blinding.
- **Database lock:** after the Principal Investigator, sponsor, statistical analyst and DM jointly sign the registration, Research-IGZ 2.0 consolidates the database allowing only queries.
- **Statistical analysis:** the statistician, through his/her profile and digital signature, can consult the database validated by the monitor, generating the spreadsheets that will be analyzed during and at the end of the study.
- **eCRF File:** Each participant's eCRF will generate PDF electronic file for preservation.
- **Research-IGZ closure:** after completing the final report and project documents, a source/object file is generated allowing only queries on the Research-IGZ platform.

The external data transmission contract needs to be signed for DMP to manage the external data.

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MEDICAL CODING

Participants' clinical findings should be reported using MedDRA (version 23.0), the drug combination adopts the WHO ATC for additional coding versus the Anatomic Therapeutic Chemical (ATC) classification.

DATA COLLECTION

All data collected must be registered in the source documents and in the eCRF in an ALCOA-C manner: **A**ttributable, **L**egible, **C**ontemporary, **O**riginal, **A**ccurate and **C**omplete. Data, such as AE reports, clinical examination results, among others, must be recorded when they occur, or as soon as possible, in a detailed manner that reflects reality. Data should be traceable, so that any changes do not obscure the original data.

The study monitor, designated by the sponsor, will be responsible for reviewing the eCRF data against the source data for completeness and accuracy. If there is a discrepancy between the data, an action plan should be developed and implemented by the research site staff.

PARTICIPANT ID

The Principal Investigator agrees to complete a participant identification and inclusion record to allow easy identification of each participant during and after the study. Research participants will be identified in the eCRF, and in any other reports or communications, only by their identification number (registration number or PID, for randomised participants). The document will be reviewed by the sponsor's contact person for completeness. The record of the participant's ID number and inclusion will be treated as confidential and will be filed by the Principal Investigator in the study file. To ensure the confidentiality of the participant, no copies will be made.

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SOURCE DOCUMENTS

The source documents of the study are all the information, original records of clinical findings, observations and results of laboratory tests and imaging necessary for the evaluation proposed in this study. The exams and data recorded directly in the eCRF will be considered the source of raw data of the study.

The research site will maintain records of participants and imaging examinations appropriate for this study in accordance with ICH E6, Section 4.9 and in accordance with regulatory and institutional requirements for protecting participant confidentiality.

DATA COLLECTION FORM

All data collected for the purposes of this study will be entered into an electronic data collection form (Case Report Form - eCRF) developed specifically for the study. These will be accessed in a restricted manner by professionals delegated by the sponsor and Principal Investigator for the study, being stored in the cloud, with adequate measures for data backup and system stability.

Data entry into the eCRF should be done by the Principal Investigator or a designated team member, and for this function, at all stages foreseen in the study.

Data regarding the results of each participant's examination,where applicable, will be transcribed into the eCRF by the Principal Investigator and/or delegated team from the source documents as information becomes available.

The Sponsor will be responsible for data management of this study, including checking data quality. All entries, corrections and changes to the eCRF must be made by professionals who will ensure the confidentiality of the participant as well as the security and confidentiality of the data throughout the study.

After completion, all data will be securely transmitted to the sponsor within the timeframe agreed upon between the sponsor and the site. After all data

collected and monitored, the eCRF will be considered closed and will be locked for changes.

PROTOCOL DEVIATIONS

The procedures of this study shall be performed as described in the protocol, taking care that protocol deviations do not occur. A protocol deviation is any non- compliance with the principles of GCP and with the study protocol. The Principal Investigator must assess each deviation that occurs during the conduct of the study for non-compliance with GCP regulations, national ethical and regulatory regulations.

Deviation may occur either on the part of the participant, the Principal Investigator, or the research site staff. **Once deviation is identified, corrective actions should be developed by the facility and implemented immediately.** Any deviation and appropriate corrective actions should be recorded, justified and documented in the partial, if applicable, and final study reports, including supporting documentation.

Deviations can be problems of adherence to the protocol, including failure to obtain the TCLE, non-compliance with eligibility criteria, incorrect performance of tests or analyses, failure in the correct administration of study drugs, among others.

PROTOCOL AMENDMENTS

Amendments to the protocol should be approved and signed in the same manner as the protocol. The Principal Investigator should not implement any protocol amendments without first discussing the changes with the sponsor and obtaining documented approval from ethical and regulatory authorities, except when necessary to eliminate immediate risks to the participant or when the changes involve only logistical or administrative aspects of the trial (e.g., changing personnel, telephone numbers).

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DATA SECRECY AND PROTECTION

It is the responsibility of the Principal Investigator, or a professional delegated to him/her, to collect, store, and disclose the study participants' information in an anonymous manner. All information should be traceable. **All study documents, including letters of approval from the REC, source documents, SCRs and other documents must be archived by the Principal Investigator for a period of 5 years in accordance with national regulations.**

Only staff members assigned to the study should have access to the data collected, in addition to the people designated by the sponsor, so that the confidentiality and security of the research participants' data are guaranteed. Files will be named with the inclusion of a participant ID. **Only the system and the physical security report will**

know the relationship between the participant's name and her corresponding number. De-identified data will be stored in the cloud and on secured computers.

All personal data collected relating to participants, Principal Investigator or any person involved in the trial who may be included in the sponsor's databases must be handled in accordance with applicable laws and regulations, including the General Data Protection Regulation.

The data collected should be adequate, relevant and not excessive in relation to the purposes for which they are collected. Each category of data should be properly justified and in line with the purpose of the study.

MONITORING

A representative of the sponsor or his/her designee will visit the investigator periodically for the purpose of monitoring the progress of the trial in accordance with the protocol, GCP and local regulations. Non-adherence to the protocol, GCP and local regulations will be documented and corrective actions will be implemented if

necessary. **It is the investigator's responsibility to be present or available for clarification during monitoring visits.**

The sponsor may use a combination of monitoring techniques in this trial: **central monitoring, remote monitoring, and in-person monitoring at the research site.** The sponsor will conduct monitoring visits at the research site as frequently as required according to the clinical monitoring plan to ensure consistency between eCRFs and source documents. **During these routine visits, all data pertaining to a trial research participant will be made available to the trial monitor.** Discrepancies will be resolved in accordance with GCP principles. The monitor will record the dates of visits in a research site visit log that will be maintained at the research site. The first post-initiation visit will be conducted as soon as possible after inclusion has begun.

AUDIT

Representatives of the sponsor may visit the research site at any time during or after completion of the trial to conduct a trial audit in accordance with regulatory guidelines and company policy. These audits will require access to all trial records, including source documents, for inspection. However, the privacy of the research participants should be respected. **The principal investigator and research site staff are responsible for being present and available for consultation during routinely scheduled research site audit visits conducted by the sponsor or its designees.**

Similar audit procedures may also be conducted by agents of any regulatory agency, either as part of a national GCP compliance program or to review the results of this trial in support of a regulatory submission. The principal investigator should notify the sponsor immediately if contacted by a regulatory agency regarding an upcoming inspection.

QUALITY CONTROL AND QUALITY ASSURANCE

In order to ensure the quality of the trial, the sponsor, ORPC and Principal Investigator should discuss and compose the clinical research plan together prior to

the formal trial and conduct GCP training for the staff participating in the trial to ensure the safety of the participants and the efficacy of the clinical trial data, data and safety monitoring should be established.

The research institution should manage study medication, including receipt, storage, distribution and recycling in accordance with standard operating procedures.

According to the ICH GCP guidelines, the necessary steps should be discussed in the design and implementation phases of the trial to ensure the accuracy, consistency, completeness and credibility of the data collected. All observed results and abnormal findings in the clinical trial should be verified and recorded at the time of their occurrence to ensure reliability of the data. All types of instruments, equipment, reagents, standards, among others, used during the clinical trial should be calibrated and have strict quality standards to ensure their functioning under normal conditions.

The Principal Investigator, or someone delegated by him, should fill in the information required by the protocol in the original medical record, and the monitor should check whether the eCRF filing is complete and accurate or not, and then direct the research site staff to make corrections and additions if necessary. Drug administration authorities and the sponsor may entrust inspectors to conductsystematic inspection of activities and documents related to the clinical trial to assess whether the trial is conducted in accordance with the requirements of the trial protocol, SOPs and relevant regulations or not and whether the experimental data are recorded timely, truthfully, accurately and whether they are complete or not. The audit shall be performed by personnel not directly involved in the clinical trial.

DATA ARCHIVING AND SAFEKEEPING

The Principal Investigator shall keep all relevant information of the study for at least 5 (five) years after its end (Resolution CNS/MS no. 466/12). Access to this file will

be controlled, being the responsibility of the Principal Investigator and the sponsor the due maintenance of the conditions that allow the adequate storage of the study information.

Prior to the removal or destruction of study documents, the research facility must inform the sponsor in writing of its intentions. Only after obtaining written permission from the sponsor may the Principal Investigator remove or destroy the study documents.

The study file may be checked by the Principal Investigator and research team, monitor and regulatory agents to ensure that all necessary documents have been obtained prior to the start of the study and have been kept up to date during the study.

11. STATISTICAL ANALYSIS

SAMPLE SIZE ESTIMATION

This study is a randomized, parallel, double-blind, PLACEBO-controlled clinical trial, with the primary endpoint being the "Proportion of patients hospitalized during the study until day 28, according to the WHO ordinal scale of clinical progression (Jun/2020), Score 4 to 10. [Period: until D15, D21 and D28, approximately 14-30 days]".

Based on the article "Hospitalization and mortality associated with SARS-CoV-2 viral clades in COVID-19[26]", the hospitalization rate occurred in 18.5% of participants who were infected with SARS-CoV-2. To consider the test drug effective, the researcher adopts the intervention group's hospitalization rate reduction of 5.5%. That said, using the sample calculation formula for studies with the variable proportion, using the R software and the TrialSize package, we calculated the sample size according to the following command:

[26] https://doi.org/10.1038/s41598-021-82850-9

TrialSize: Two Sample Proportion.NIS (alpha, beta, p1, p2, k, delta, margin)

Parameters						
alfa	**beta**	**p1**	**p2**	**k=n1/n2**	**delta=p1-p2**	**Margin**
0.05	0.05	0.055	0.185	1	0.13	0.00

The sample size necessary for the IGZ-1 protocol is 260 participants, with 130 participants in each group. Considering a 20% increase to make up for eventual losses of follow-up, we have a total of 312 patients.

STATISTICAL ANALYSIS: STUDY STRATEGY DEFINITIONS

- Complete analysis set (FAS): a set of patients who were randomized and received at least one oral dose of the study drug.
- Per protocol set (PPS): A set of data generated by a subset of clinical trial subjects whose protocol compliance was strict, with no significant deviation from the trial protocol, to ensure that the data are statistically relevant for answering the primary endpoint. The PPS analysis shall be used for the primary efficacy indicator.
- Safety Data Set (SS): A set of participants who received at least one treatment, and have actual data recorded by the safety index after treatment. The SS analysis will be used for the incidence of adverse reactions.
- For the treatment of missing values the Expected Maximization technique will be used, in which an interactive process of replacement of missing data based on the mean of the items and the covariance pattern of the variables is performed until the convergence of the results.

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STATISTICAL ANALYSIS PLAN

Preliminarily, the statistical analysis will follow the following topics:

- Quantitative variables describe the mean value, standard deviation, median, minimum value, maximum value, lower quartile (Q1), upper quartile (Q3) to be calculated, and categorical variables describe the frequency and percentage of cases;
- The comparison of the two groups in general conditions will be analyzed with appropriate methods according to the types of indicators/variables. Student's t-test or Mann-Whitney test will be used to compare groups for quantitative data. Chi-square test or Fisher's exact test will be used for categorical data. The tests will be detailed in the topics below;
- The selected cases should be grouped and at the end of the study determine the three sets of analysis data (FAS, PPS, SS).
- A significance level of 5% will be adopted.

FOR THE PRIMARY OUTCOME:

- Proportion of patients hospitalized during the study until day 28, according to the WHO ordinal scale of clinical progression (Jun/2020), Score 4 to 10.[Period: until D15, D21 and D28, approximately 14-30 days]. Hospitalized participants will be considered if the score is between 4 and 10 during the study until day 28.

VARIABLE	TYPE
HOSPITALIZATION	CATEGORY (4 a 10/<4)
PEARSON CHI-SQUARE TEST	

  - Those who worsen at any time, up to D28, for hospitalization will be considered hospitalized participants, remembering that participants who worsen for hospitalization will be discontinued from the drug.

- Subgroup analyzes will be performed by comorbidities: cardiovascular disease, hypertension and diabetes[27,28].
  - For each SUBGROUP variable (presence of cardiovascular disease, hypertension or diabetes), a Binary Logistic Regression will be performed with the dependent variable HOSPITALIZATION and independent variables of the Study Allocation Group (Control/AZVUDINE) and the SUBGROUP variable (presence of disease cardiovascular disease, hypertension or diabetes).
- Subgroup analyzes will be performed by time of use of AZVUDINE
  - For the SUBGROUP analysis (time of use of AZVUDINE), a Binary Logistic Regression with the dependent variable HOSPITALIZATION and independent variables of the Study Allocation Group and the time of use of AZVUDINE will be performed.

FOR THE SECONDARY OUTCOME:

- "Time for healing[29]"

VARIABLE	TYPE
HEALING TIME	CATEGORY VARIABLE (<=48h/>48h)
PEARSON'S CHI-SQUARE TEST/ FISHER'S EXACT TEST	

- Improvement in clinical status in at least one category compared to screening on the Ordinal Scale of Clinical Improvement (WHO, Jun/2020) [Period: until D15,

[27] Wang, X., Fang, X., Cai, Z., Wu, X., Gao, X., Min, J., & Wang, F. (2020). Comorbid chronic diseases and acute organ injuries are strongly correlated with disease severity and mortality among COVID-19 patients: a systemic review and meta-analysis. *Research*, *2020*.

[28] Luo, L., Fu, M., Li, Y., Hu, S., Luo, J., Chen, Z., ... & Xu, X. (2020). The potential association between common comorbidities and severity and mortality of coronavirus disease 2019: a pooled analysis. *clinical cardiology*, *43*(12), 1478-1493.

[29]According to the WHO Working Group on Characterization and Clinical Management of COVID-19 Infection (July, 2020), clinical and virological absence of infection (non-detectable RT-PCR) is suggestive of CURE for initially infected participants.

approximately 14 days][Score 1 to 3].

WHO CLINICAL PROGRESSION SCALE (JUN/2020)		
CLINICAL FRAMEWORK	**VARIABLE DESCRIPTION**	**SCORE**
Outpatient: mild disease	Asymptomatic; viral RNA detected.	1
	Symptomatic; does not require assistance.	2
	Symptomatic; needs assistance.	3

- Clinical improvement will be considered as the reduction of the score in at least one category in comparison with the screening during the period D-1 to D15.

VARIABLE	**TYPE**
IMPROVEMENT IN CLINICAL STATUS	CATEGORY (YES/NO)
PEARSON CHI-SQUARE TEST	

- Severity and duration of symptoms: fever, cough, fatigue or tiredness, breathlessness, myalgia, nasal congestion or runny nose, sore throat, headache, chills, nausea, vomiting, anosmia, ageusia; [Period: up to D15, D21 and D28, approximately 14-30 days].

VARIABLES	**TYPE**
Stuffy or runny nose	none = 0

Sore throat	smooth = 1 Moderate = 2 Severe = 3
Shortness of breath (difficulty breathing)	
Cough	
Low energy or tiredness	
Muscle or body aches	
Headache	
Chills or tremors	
Feeling hot or feverish	
Nausea (feeling like you wanted to throw up)	
How many times have you vomited in the last 24hours? **	I didn't throw up anything = 0 1-2 times = 1 3-4 times = 2 5 or more times = 3
How many times have you had diarrhea (loose or watery stools) in the last 24 hours? **	I didn't have diarrhea at all = 0 1-2 times = 1 3-4 times = 2 5 or more times = 3
Assess your sense of smell in the last 24 hours	My sense of smell is O EVEN AS USUAL = 0 My sense of smell is LESS THAN usual = 1 I have NO sense of smell = 2

Rate your sense of taste in the last 24 hours	My sense of taste is the SAME AS usual = 0 My sense of taste is LESS THAN usual = 1 I don't have sensitivity taste = 2
A VARIABLE FOR EACH SYMPTOM	CATEGORY
STATISTICAL TEST TO BE USED: PEARSON'S CHI-SQUARE TEST	

- Changes in liver and renal function baseline; [Period: until D15, D21 and D28, approximately 14-30 days]

VARIABLES	**TYPE**
ALTERED KIDNEY FUNCTION	CATEGORY (YES/NO)
ALTERED LIVER FUNCTION	CATEGORY (YES/NO)
STATISTICAL TEST TO BE USED: PEARSON CHI-SQUARE	
ONE VARIABLE FOR EACH MARKED. E.G. TGO, TGP, CREATININE ETC.	CONTINUOUS NUMBER
TESTE ESTATÍSTICO A SER UTILIZADO: TESTE T DE STUDENT/ TESTE DE MANN-WHITNEY	

- The values of laboratory tests will be compared with the baseline of the experimental group and the control group separately, in the period from D-1 to D15, D21 and D28 [approximately 14-30 days].
    - Laboratory tests performed on days D1, D3, D5, D7, D9, D13, D15, D21, D28 and D60

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VARIABLES	TYPE
ABNORMAL LABORATORY TESTS	CATEGORY (YES/NO)
STATISTICAL TEST TO BE USED: PEARSON CHI-SQUARE	
ONE VARIABLE FOR EACH MARKED. E.G. D-DIMER, TROPONIN I, FIBRINOGEN, ETC.	CONTINUOUS NUMBER
STATISTICAL TEST TO BE USED: STUDENT'S T TEST/ MANN-WHITNEY TEST	

- Time of AZVUDINE use until the second negative RT-PCR conversion [Period: until D15, approximately 14 days].

VARIABLE	**TYPE**
TIME OF AZVUDINE USE UNTIL THE SECOND NEGATIVE CONVERSION	CATEGORY VARIABLE (<=48h/>48h)
PEARSON'S CHI-SQUARE TEST/ FISHER'S EXACT TEST	

- Negative conversion time of SARS-CoV-2 viral load by RT-PCR between AZVUDINE (FNC) group and control group.

VARIABLE	**TYPE**
NEGATIVE VIRAL LOAD CONVERSION TIME	CATEGORY VARIABLE (<=48h/>48h)
PEARSON'S CHI-SQUARE TEST/ FISHER'S EXACT TEST	

- Negative conversion of SARS CoV2 by qualitative RT-PCR and/or absence of quantitative viral load, between D1 to D15 or D21 and confirming negative maintenance on D28.

VARIABLE	**TYPE**
NEGATIVE VIRAL LOAD CONVERSION	CATEGORY (YES/NO)
TESTE ESTATÍSTICO A SER UTILIZADO: QUIQUADRADO DE PEARSON	

- **Time of the first and second negative conversion of SARS-CoV-2 viral load**, relative

to the start of treatment, by RT-PCR between the AZVUDINE (FNC) group and the control group separately in the period D1 to D15, or to D21 and maintenance of negative conversion at D28 [approximately 14-30 days].

VARIABLE	TYPE
TIME OF THE FIRST AND SECOND NEGATIVE VIRAL LOAD CONVERSION	CATEGORY VARIABLE (<=48h/>48h)
PEARSON'S CHI-SQUARE TEST/ FISHER'S EXACT TEST	

- Evaluation of the number of cycles for the detection of SARS-CoV-2 viral load by RT-PCR and application of the standard curve for viral load calculation; [Period: until D15, D21 and D28, approximately 14-30 days].
- Comparison of the **number of cycles for the detection of SARS-CoV-2 viral load by RT-PCR to establish intensity of infection** during treatment, and every 48 h, between the AZVUDINE (FNC) group and the control group in the period from D1 to D15, or to D21 and negative control at D28 [approximately 14-30 days].

CTs	Viral load	
15,0	14.000,00	Multiplication phase
20,0	12.000,00	
25,0	10.000,00	
27,0	1.000,00	Resolution phase
29,0	100,00	
30,0	10,00	
30,6	Negative	

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VARIABLE	TYPE
VIRAL LOAD/CTs	DISCRETE NUMBER
STATISTICAL TEST TO BE USED: STUDENT'S T TEST/ MANN-WHITNEY TEST	
VARIABLE	**TYPE**
VIRAL LOAD DETECTION	CATEGORY (MULTIPLICATION PHASE/RESOLUTION PHASE)
STATISTICAL TEST TO BE USED: PEARSON CHI-SQUARE TEST	

- Analysis of the relationship between the calculated and/or quantified viral load and the clinical evolution of the participants in the AZVUDINE (FNC) group compared to the control group; VIRAL LOAD/CTs, SCORE, NASAL CONGESTION, GARGAGE PAIN, LACK OF AIR, CUSTOMS, FADIGUE, MIALGIA, PHALEIA, OLPHATE, AND SMELL.

CLINICAL FRAMEWORK	DESCRIPTION	SCORE
Not infected	without detection of viral RNA.	0
Outpatient: mild disease	Asymptomatic; viral RNA detected.	1
	Symptomatic; does not require assistance.	2
	Symptomatic; needs assistance.	3
Hospitalized:	Hospitalized; no oxygen	4

moderate illness	therapy.*	
	Hospitalized; oxygen support by mask or nasal catheter.	5
Hospitalized: serious illness	Hospitalized; oxygen support by NIV or high flow.	6
	Intubation and mechanical ventilation, pO2/FiO2 ≥150 ou SpO2/FiO2 ≥200.	7

VARIABLES	**TYPE**
CLINICAL OUTCOME VARIABLES	NUMERIC ORDINAL
STATISTICAL TEST TO BE USED: SPEARMAN CORRELATION COEFFICIENT	

- Drug Interactions; [Period: until D15, D21 and D28, approximately 14-30 days. **The number of AE occurrences reported and related to the drug interaction**, during the treatment period (D1 to D14) or lasting until D28 [approximately 14-30 days] will be**computed.**

- Adverse events, unexpected adverse events, and serious adverse events; [Period: until D15, D21 and D28, approximately 14-30 days]. **Frequency of AEs, EAGs, unexpected AEs, reported,** during the treatment period (D1 to D14) or lasting until D28 [approximately 14-30 days].

VARIABLES	**TYPE**
NUMBER OF ADVERSE EVENTS	DISCRETE NUMBER

NUMBER OF EXPECTED ADVERSE EVENTS	DISCRETE NUMBER
NUMBER OF SERIOUS ADVERSE EVENTS	DISCRETE NUMBER
STATISTICAL TEST TO BE USED: STUDENT'S T TEST/ MANN-WHITNEY TEST	

VARIABLES	**TYPE**
ADVERSE EVENTS	CATEGORY (YES/NO)
UNEXPECTED ADVERSE EVENTS	CATEGORY (YES/NO)
SERIOUS ADVERSE EVENTS	CATEGORY (YES/NO)
STATISTICAL TEST TO BE USED: PEARSON CHI-SQUARE	

- To assess tolerability of AZVUDINE (FNC) at 5mg/day for up to 14 days: [Period: until D15, approximately 14 days. **The number of refusals of the participant to take the experimental medication** during the treatment period (D1 to D14) will be **computed;**

- Proportion of aggravations related to comorbidities [Cardiovascular disease, hypertension, diabetes]; [Period: until D15, approximately 14 days][30,31].

VARIABLES	**TYPE**
AGGREGATE	DISCRETE NUMERIC SCORE
CARDIO VASCULAR DISEASE	CATEGORY (YES/NO)
HIGH BLOOD PRESSURE	CATEGORY (YES/NO)
DIABETES	CATEGORY

[30]Wang, X., Fang, X., Cai, Z., Wu, X., Gao, X., Min, J., & Wang, F. (2020). Comorbid chronic diseases and acute organ injuries are strongly correlated with disease severity and mortality among COVID-19 patients: a systemic review and meta-analysis. *Research*, *2020*.

[31]Luo, L., Fu, M., Li, Y., Hu, S., Luo, J., Chen, Z., ... & Xu, X. (2020). The potential association betweencommon comorbidities and severity and mortality of coronavirus disease 2019: a pooled analysis. *clinical cardiology*, *43*(12), 1478-1493.

	(YES/NO)
STATISTICAL TEST TO BE USED: PEARSON CHI-SQUARE	

- Aggravations occurring in the post-treatment period, up to 60 days or outcome. Period: until D60 [approximately 60 days].

VARIABLE	TYPE
AGGREGATE	DISCRETE NUMERIC SCORE
STATISTICAL TEST TO BE USED: STUDENT'S T TEST/ MANN-WHITNEY TEST	

- **The number of participants who completed treatment**, during the treatment period (D1 to D14) and returned for follow-up visits D28 and D60 will be **computed;**
- **The time of AZVUDINE use from D1 to the second negative conversion of viral load by RT-PCR** during the treatment period (D1 to D14) will be **computed;**
- **The amount of early termination due to withdrawal** during the treatment period (D1 to D14) will be **computed;**
- **The number of participants who completed treatment** but did not return for follow-up visits D28 and D60 will be **computed.**

VARIABLES	TYPE
ADHERED TO TREATMENT	CATEGORY (YES/NO)
ABANDONED TREATMENT	CATEGORY (YES/NO)
STATISTICAL TEST TO BE USED: STUDENT'S T TEST/ MANN-WHITNEY TEST	
TOTAL TIME OF USE OF AZVUDINE	DISCRETE NUMBER
STATISTICAL TEST TO BE USED: PEARSON CHI-SQUARE	

- Relation of age and comorbidity with disease worsening will be stratified statistically

and for this calculation through biserial point correlation.

VARIABLES	TYPE
AGGREGATE	DISCRETE NUMERIC SCORE
COMORBITY	CATEGORY (YES/NO)
STATISTICAL TEST TO BE USED: BISECTORIAL POINT CORRELATION	

MODEL AND OPERATIONAL CHARACTERISTICS

The sample size is 312 participants, including 156 in the experimental group and 156 in the control group in a 1:1 ratio between the treatment and control groups, considering a 20% dropout.

From the approval of the study by the Research Ethics Committee and ANVISA, it is estimated the period of approximately 6 months for its completion.

During the study, the Independent Data and Safety Monitoring Committee (IMSMC) will carry out safety monitoring (adverse events, clinical, statistical, pharmacological and toxicological issues) and, if necessary, experts in relevant areas may also be invited to attend the IMSMC meeting.

At the end of the study, the participants' data will be analyzed and submitted to statistical analysis of the results according to the pre-established methodology.

12. ETHICAL AND REGULATORY CONSIDERATIONS AND GOOD CLINICAL PRACTICE

This protocol was prepared and will be conducted in accordance with the standards established by the ICH Guidelines for Good Clinical Practice GCP/ICH - ICH Topic E6 (R2) (2016), Americas Document (2005), Resolution No. 466/12 of the National Health Council and related, as well as the RDC No. 09 of February 2015 (ANVISA) and other applicable regulatory requirements. The data obtained will be used only for purposes determined in this research, keeping the confidentiality of data and

results obtained ensured by controlled access of persons responsible for its evaluation and execution.

REGULATORY CONSIDERATIONS

Prior to initiation of the trial, the Principal Investigator shall obtain written consent from the national regulatory authorities regarding the trial protocol and other documents that may be submitted. During the study, if there are further changes in the study protocol, written approval from ANVISA must be obtained again.

INDEPENDENT DATA AND SAFETY MONITORING COMMITTEE (CIMS)

In order for the trial to meet to the greatest extent possible the scientific and ethical standards, to ensure the safety and well-being of the participants and the scientific validity and integrity of the data, a CIMS has been established for the trial to review the safety data, perform emergency review and assessment on safety-related issues. The members of the CIMS shall assess adverse events, clinical, statistical, pharmacological and toxicological issues and, if necessary, experts in relevant fields may also be invited to attend the CIMS meeting.

The CIMS will be responsible for assessing the safety of the investigational medicinal product. The CIMS may discontinue the study on safety grounds.

Throughout the process of this trial, CIMS will continuously monitor the safety data, propose to the principal investigator and sponsor to arrange timely data discussion meeting, analyze the safety indicators and provide suggestions to the principal investigator and sponsor for the follow-up trial design according to the results of statistical analysis. This committee will also conduct regular meetings or teleconferences according to the CIMS regulations, and the committee's recommendations include terminating the trial for safety, modifying the protocol or suspending the trial until more supporting information is available, with the sponsor having priority in the final decision.

The CIMS regulations for working procedures can be found in **ANNEX 3.**

ETHICAL CONSIDERATIONS AND RESEARCH ETHICS COMMITTEE (CEP)

According to the Resolution of the National Health Council No. 466/2012, the Operational Standard No. 001/2013 and complementary, research participants will be assured full freedom to refuse to participate or to withdraw their consent at any stage of the research, without any penalty, and the maintenance of confidentiality and privacy during all phases of the research.

Prior to the initiation of the study, the protocol, the informed consent form, as well as other relevant documents, will be submitted to the Research Ethics Committee for approval. The study may not be initiated until all relevant approvals have been obtained.

The research project will be registered in Plataforma Brasil for ethical review by the Research Ethics Committee (CEP) of Faculdade de Medicina de Campos, accredited by CONEP - CNS/MS. The referred research project cannot be initiated before the approval by CEP.

PROCESS FOR OBTAINING THE INFORMED CONSENT(ICF)

The Principal Investigator, or person delegated by him/her, will explain to each participant the nature of the study, objectives, justification, methods, and possible risks and benefits with their participation. Participants will be informed that they have the freedom to refuse to participate and may withdraw their consent at any time and for any reason, without any prejudice. The Principal Investigator, or person delegated by him/her, will be available to answer questions and clarify any doubts. The research participant will have enough time to read the TCLE, ask questions and talk to friends and family, if they feel the need, before deciding to participate in the study. The research participant will be informed that his/her privacy and the confidentiality of his/her data will be assured by the Principal Investigator and team and will be given the assurance that the data obtained from the research will be used exclusively for the

intended purpose. Any publication arising from this research will be carried out in a grouped form, not allowing the individual identification of the participants.

The Term of Consent **(APPENDIX 4)** will be presented in two copies of equal value, and must be initialed on all its pages and signed, at the end, by the person invited to participate in the research, or by his/her legal representative, as well as by the Principal Investigator, or by the person(s) delegated by him/her, before his/her participation in this study.

If the participant or legally acceptable representative is unable to read or write, an impartial witness must be present for the entire informed consent process (which includes reading and explaining all written information) and must personally date and sign the ICF after verbal consent from the participant or legally acceptable representative.

The Term of Consent shall be revised whenever changes occur in the study procedures or when new information that may affect the disposition of the potential participant becomes available. The Term of Consent that is updated after its final approved version must be submitted to a new approval by the REC. Participants must provide new consent for the most current version of the Term of Consent.

REIMBURSEMENT

The research participants will be entitled to reimbursement for their expenses and those of their companions, when necessary, such as: food, transportation, laboratory tests, contraceptive methods and other necessary costs during the conduction of the study, in compliance with the requirements established in items II.21 and IV.3.g of Resolution No. 466/2012 of the CNS.

PARTICIPANT ASSISTANCE

The sponsor and the Principal Investigator assure immediate, full and free assistance for complications and damages directly or indirectly related to the

participation in the study, for as long as necessary. Furthermore, all research participants who may suffer any type of damage because of their participation in this research are entitled to compensation, in compliance with the requirements established in items II.3, IV.7 and V.6 of Res. no. 466/2012.

CONFIDENTIALITY AND DISSEMINATION OF RESEARCH RESULTS

Toda a equipe envolvida no estudo deve manter todas as informações fornecidas pelo patrocinador estritamente confidenciais, assim como autoridades éticas e regulatórias. As informações não devem ser divulgadas a terceiros sem permissão por escrito.

All staff involved in the trial should keep all information provided by the sponsor and ethical and regulatory authorities strictly confidential. Information should not be disclosed to third parties without written permission.

All data and experimental results of this study are joint property of the Principal Investigator and the sponsor. In compliance with the CNS Resolution No. 466/2012 and CNS Operational Standard No. 001 of 2013 of the National Health Council, of the Ministry of Health and other complementary of the CEP/CONEP System, the research results will be published, whether favorable or not, with due credit to the Principal Investigator and technical staff involved and attached to Plataforma Brasil in the form of a Research Report.

The publication of data in specialized scientific journal or in events of the areas (congresses, symposiums, meetings) should be done in a grouped way to ensure the confidentiality of the data and the privacy of the participants meeting the regulatory requirementswith due credits to the authors.

The results of the study will be disseminated among the research participants and institutions where the data was obtained. The rights arising from all results and any discovery or innovation arising from the study are reserved solely and exclusively to the sponsor.

This protocol and its results will be registered in the platform www.clinicaltrials.gov.

RESPONSIBILITY OF THE PRINCIPAL INVESTIGATOR

The Principal Investigator is responsible for conducting the trial in compliance with the protocol, GCP, and ethical and regulatory requirements, and is responsible for personally supervising all staff involved in the clinical trial. The Principal Investigator may delegate tasks assigned to him/her, but not responsibility. The Principal Investigator shall allow and cooperate with monitoring, audits and inspections.

The Principal Investigator shall read and understand the IB, including potential risks and AEs of the study medicinal products, and ensure that adequate medical care is provided to subjects free of charge as a result of any AEs relating to the clinical trial. The Principal Investigator shall record and report to the sponsor any AEs that occur during the trial according to national regulations.

In the event of premature termination or suspension of the clinical trial for any reason, the Principal Investigator shall immediately inform the trial subjects, ensuring the appropriate follow-up, safety and well-being of the trial subjects.

It is the responsibility of the Principal Investigator to ensure that protocol changes are made only after written approval from the sponsor and ethical and regulatory authorities, except to protect the safety, rights or welfare of participants.

It is the responsibility of the Principal Investigator, or professional delegated by him/her, to register, store and disclose the information from this study, as well as to ensure the quality and traceability of the data. **All the study documents, including the conscientious opinion issued by the REC, source documents, ANVISA approval and others, should be archived by the Principal Investigator for a period of 5 years, following the national regulations (Resolution no. 466/2012 - CNS/MS and RDC no. 9/2015 - ANVISA), or for a longer period, if agreed with the sponsor.**

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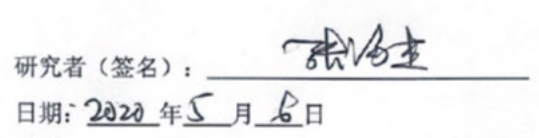
The principal investigator and sponsor should sign on the protocol signature page to evidence that both have agreed to the contents of the protocol and that they will conduct and implement the trial in accordance with the protocol.

RESPONSIBLE FOR THE AZVUDINE STUDY

The physician responsible for AZVUDINE in China is Professor Fujie Zhang, who is director and professor of medicine Clinical and Research Center of Infectious Diseases Beijing Ditan Hospital, Capital Medical University.

The signature of Dr.Zhang in Investigator's Brochure of AZVUDINE Tablets in China is as follows:



The responsible Physician for AZVUDINE in Brazil is:

Paula Gebe Abreu Cabral, CRM: 52.52.956-01

Contact: (22) 98126 6602

The Principal Investigator of the IGZ-2 study is:

Carlos Augusto de Araújo Tavares, CRM: 52 2766

Contact (22) 999737500

TIME FRAME FOR COMPLETION OF THE STUDY

The estimated time from screening (Day -1 or Day 1) to the end of the study (last follow-up visit D60) for a participant is approximately 60 days. As a total study deadline, it is estimated approximately 6 months, after study approval by the Research Ethics Committee (CEP) and ANVISA, for completion and delivery of the Final Report.

REFERENCES

Abdullah, S. F., & Sharquie, I. K. (2020). Sars-cov-2: A piece of bad news. *Medeniyet Medical Journal*, *35*(2), 151–160. https://doi.org/10.5222/MMJ.2020.82584

Caly, L., Druce, J. D., Catton, M. G., Jans, D. A., & Wagstaff, K. M. (2020). The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Research*, *178*, 104787. https://doi.org/10.1016/j.antiviral.2020.104787

Carroll, S., & Olsen, D. (2006). Nucleoside Analog Inhibitors of Hepatitis C Virus Replication. *Infectious Disorders - Drug Targets*, *6*(1), 17–29. https://doi.org/10.2174/187152606776056698

Chibber, P., Assim, S., Ahmed, I., & Iqbal, N. (2020). *Advances in the possible treatment of COVID-19: A review*. *January*.

Choy, K.-T., Wong, A. Y.-L., Kaewpreedee, P., Sia, S. F., Chen, D., Hui, K. P. Y., Chu, D. K. W., Chan, M. C. W., Cheung, P. P.-H., Huang, X., Peiris, M., & Yen, H.-L. (2020). Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. *Antiviral Research*, *178*, 104786. https://doi.org/10.1016/j.antiviral.2020.104786

Domingo, P., Mur, I., Pomar, V., Corominas, H., Casademont, J., & de Benito, N. (2020). The four horsemen of a viral Apocalypse: The pathogenesis of SARS-CoV-2 infection (COVID-19). *EBioMedicine*, *58*, 102887. https://doi.org/https://doi.org/10.1016/j.ebiom.2020.102887

Drożdżal, S., Rosik, J., Lechowicz, K., Machaj, F., Kotfis, K., Ghavami, S., & Łos, M. J. (2020). FDA approved drugs with pharmacotherapeutic potential for SARS-CoV-2 (COVID-19) therapy. *Drug Resistance Updates*, *53*, 100719. https://doi.org/10.1016/j.drup.2020.100719

Ezzikouri, S., Nourlil, J., Benjelloun, S., Kohara, M., & Tsukiyama-Kohara, K. (2020). Coronavirus disease 2019—Historical context, virology, pathogenesis, immunotherapy, and vaccine development. *Human Vaccines and Immunotherapeutics*, *00*(00), 1–9. https://doi.org/10.1080/21645515.2020.1787068

Fehr, A. R., & Perlman, S. (2015). Coronaviruses: An Overview of Their Replication and Pathogenesis. In *Coronaviruses: Methods and Protocols* (pp. 1–23). https://doi.org/10.1007/978-1-4939-2438-7_1

Götz, V., Magar, L., Dornfeld, D., Giese, S., Pohlmann, A., Höper, D., Kong, B.-W., Jans, D. A., Beer, M., Haller, O., & Schwemmle, M. (2016). Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. *Scientific Reports*, *6*(1), 23138. https://doi.org/10.1038/srep23138

Jordan, P. C., Stevens, S. K., & Deval, J. (2018). Nucleosides for the treatment of respiratory RNA virus infections. *Antiviral Chemistry and Chemotherapy*, *26*, 204020661876448. https://doi.org/10.1177/2040206618764483

Ministério da Saúde. (2020a). *Coronavírus: o que você precisa saber e como prevenir o contágio*. https://coronavirus.saude.gov.br/

Ministério da Saúde. (2020b). *Coronavírus Brasil*. https://covid.saude.gov.br/

Ministério da Saúde. (2020c). *Orientações para manejo de pacientes com Covid-19*. https://saude.gov.br/images/pdf/2020/June/17/Covid19-Orienta----esManejoPacientes.pdf

MS/SCTIE. (2020). *DIRETRIZES PARA DIAGNÓSTICO E TRATAMENTO DA COVID-19*. Ministário Da Saúde - Secretaria de Ciência, Tecnologia, Inovação e Insumos Estratégicos Em SaúdeTecnologia, Inovação e Insumos Estratégicos Em Saúde. https://portalarquivos.saude.gov.br/images/pdf/2020/May/08/Diretriz-Covid19-v4-07-05.20h05m.pdf

Nitulescu, G. M., Paunescu, H., Moschos, S. A., Petrakis, D., Nitulescu, G., Ion, G. N. D., Spandidos, D. A., Nikolouzakis, T. K., Drakoulis, N., & Tsatsakis, A. (2020). Comprehensive analysis of drugs to treat SARS-CoV-2 infection: Mechanistic insights into current COVID-19 therapies (Review). *International Journal of Molecular Medicine*, *46*(2), 467–488. https://doi.org/10.3892/ijmm.2020.4608

Prefeitura de Campos. (2020). *Infoletim NPºP 26/2020*. SMS/VIG EM SAÚDE/VIGILÂNCIA EPIDEMIOLÓGICA. https://campos.rj.gov.br/newdocs/1608982625InformeEpidemologicoCampos26.pdf

Pruijssers, A. J., & Denison, M. R. (2019). Nucleoside analogues for the treatment of coronavirus infections. *Current Opinion in Virology*, *35*, 57–62. https://doi.org/10.1016/j.coviro.2019.04.002

Ren, Z. et. al. A Randomized, Open-Label, Controlled Clinical Trial of AZVUDINE Tablets in the Treatment of Mild and Common COVID-19, a Pilot Study. Adv. Sci. 2020, 7, 2001435. https://doi.org/10.1002/advs.202001435

Romano, M., Ruggiero, A., Squeglia, F., Maga, G., & Berisio, R. (2020). A Structural View of SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping. *Cells*, *9*(5), 1267. https://doi.org/10.3390/cells9051267

Sun, L., Peng, Y., Yu, W., Zhang, Y., Liang, L., Song, C., Hou, J., Qiao, Y., Wang, Q., Chen, J., Wu, M., Zhang, D., Li, E., Han, Z., Zhao, Q., Jin, X., Zhang, B., Huang, Z., Chai, J., … Chang, J. (2020). Mechanistic Insight into Antiretroviral Potency of 2′-Deoxy-2′-β-fluoro-4′-azidocytidine (FNC) with a Long-Lasting Effect on HIV-1 Prevention. *Journal of Medicinal Chemistry*, *63*(15), 8554–8566. https://doi.org/10.1021/acs.jmedchem.0c00940

Tay, M. Y. F., Fraser, J. E., Chan, W. K. K., Moreland, N. J., Rathore, A. P., Wang, C., Vasudevan, S. G., & Jans, D. A. (2013). Nuclear localization of dengue virus (DENV) 1–4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin. *Antiviral Research*, *99*(3), 301–306. https://doi.org/10.1016/j.antiviral.2013.06.002

Wagstaff, K. M., Sivakumaran, H., Heaton, S. M., Harrich, D., & Jans, D. A. (2012). Ivermectin is a specific inhibitor of importin α/β-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochemical Journal*, *443*(3), 851–856. https://doi.org/10.1042/BJ20120150

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W., & Xiao, G. (2020). Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Research*, *30*(3), 269–271. https://doi.org/10.1038/s41422-020-0282-0

Wang, R.-R., Yang, Q.-H., Luo, R.-H., Peng, Y.-M., Dai, S.-X., Zhang, X.-J., Chen, H., Cui, X.-Q., Liu, Y.-J., Huang, J.-F., Chang, J.-B., & Zheng, Y.-T. (2014). AZVUDINE, A Novel Nucleoside Reverse Transcriptase Inhibitor Showed Good Drug Combination Features and Better Inhibition on Drug-Resistant Strains than Lamivudine In Vitro. *PLoS ONE*, *9*(8), e105617. https://doi.org/10.1371/journal.pone.0105617

WHO. (2020a). *Clinical management of COVID-19*. World Health Organization. https://www.who.int/publications/i/item/clinical-management-of-covid-19

WHO. (2020b). *Coronavirus disease (COVID-19) - Situation Report – 204*. World Health Organization. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200811-covid-19-sitrep-204.pdf?sfvrsn=1f4383dd_2

WHO. (2020c). *COVID 19 Experimental Treatments*. https://www.who.int/docs/default-source/coronaviruse/covid-classification-of-treatment-types-rev.pdf?sfvrsn=5b90b2f2_1&download=true

WHO. (2020d). *Q&A on coronaviruses (COVID-19)*. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/q-a-coronaviruses

WHO. (2020e). *WHO Coronavirus Disease (COVID-19) Dashboard | WHO Coronavirus Disease (COVID-19) Dashboard*. World Health Organization. https://covid19.who.int/

Zhand, S., Saghaeian Jazi, M., Mohammadi, S., Tarighati Rasekhi, R., Rostamian, G., Kalani, M. R., Rostamian, A., George, J., & Douglas, M. W. (2020). COVID-19: The Immune Responses and Clinical Therapy Candidates. *International Journal of Molecular Sciences*, *21*(15). https://doi.org/10.3390/ijms21155559

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ANNEXES

ANNEX 1 –GUIDELINES FOR DIAGNOSIS AND TREATMENT OF COVID-19 - April 6, 2020, Secretariat of Science, Technology, Innovation and Strategic Health Inputs - SCTIE

ANNEX 2 –Data Management Plan

ANNEX 3 –CIMS Rules of Procedure - V2.0_19.01.2021

ANNEX 4 – Informed Consent Form (ICF)

ANNEX 5 - Hope-4 Partial Data Report on the Efficacy and Safety of AZVUDINE

ANNEX 6–Contraception

ANNEX 7 –Assessing COVID19 realated symptoms in outpatients subjects in clinical trials – Translated

ANNEX 8 – Statistical Analysis Plan

ANNEX 9 – Medicinal Product Development Plan