Master Protocol:

A Phase 2, Randomized, Double-blind, Placebo-controlled Clinical Trial Evaluating the Safety and Efficacy of RESOMELAGON in Patients With Dengue Infection (RESOVIR-2)

Short title: Safety and efficacy of RESOMELAGON in dengue infection

Acronym: RESOVIR-2

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Privacy Statement

This document contains confidential information that should not be disclosed to anyone other than the Investigator's Team, host organization, members of the Research Ethics Committee and Regulatory Authorities, and the company Synact Pharma, which donated the drug for this study, unless authorized to do so.

Investigator's Agreement and Declaration of Interests

"The undersigned has read and understood the protocol of the study detailed above and:

- agrees to conduct the study according to protocol.
- agrees to abide by the principles of the *International Conference on Harmonization Tripartite Guideline on Good Clinical Practice*.
- declares that there is no conflict of interest, in accordance with the current version of the Declaration of Helsinki"



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1. SYNOPSIS

Study title	A Phase 2, Randomized, Double-blind, Placebo-Controlled Study Evaluating the Safety and Initial Efficacy of RESOMELAGON in Patients With Dengue Infection (Resovir-2).
Reasons	The complication of dengue follows the peak of viremia and is associated with increased activation of the immune response. Proof-of-concept studies in animal models of dengue infection have shown that blocking certain aspects of the inflammatory response or the administration of pro-resolving molecules is beneficial in experimental dengue. In these experiments, the administration of certain anti-inflammatory or pro-resolution molecules decreases disease severity and aspects of the inflammatory response, including changes in vascular permeability. We have previously shown that treatment with RESOMELAGON decreased lung inflammation and outcome in animal models of beta coronavirus infection (including by SARS-CoV-2) and decreased disease severity in COVID-19 patients, even in the presence of glucocorticoid therapy. Preclinical experiments also showed that RESOMELAGON decreased disease severity in an animal model of dengue infection and decreased cytokine release by human PBMC infected with dengue virus. RESOMELAGON is safe in healthy individuals, those with rheumatoid arthritis, and patients with COVID-19. Further studies are needed to evaluate the safety and efficacy of RESOMELAGON in patients with dengue.
Study design	Randomized, placebo-controlled, double-blind trial
Study patients	**Adult patients with early arboviral infection**
Inclusion and exclusion criteria	**Inclusion Criteria:** **1. Participants aged 18 to 65 years;** **2. Participant is able to understand study procedures and provide informed consent;** **3. Presents more than 36 and less than 84 hours after the onset of symptoms;** **4. Presents with symptoms consistent with dengue infection with a positive antigen test or positive PCR test. Suspicion of dengue is given by the report of fever and at least 2 other signs or symptoms of the disease, including myalgia, arthralgia, headache or conjunctivitis.** **Exclusion Criteria**: **1. Has any comorbidity that is perceived as decompensated by the investigator;**

	2. **The following significant laboratory abnormalities discovered at screening would lead to participant discontinuation** ● **Hemoglobin <10g/dl** ● **Platelet count <50,000/ul** ● **ALT> 3x ULN** ● **Total bilirubin> 1.5 x ULN** ● **GFR <60mls/min/1.73m2** 3. **Contraindications or known hypersensitivity to RESOMELAGON ;** 4. **Presents as dengue with warning signs# or severe dengue* at inclusion;** 5. **Currently participating in another clinical drug trial;** 6. **Clinical evidence of another infection that may explain the current symptoms;** 7. **Pregnant women or women actively trying to achieve pregnancy.** ***Severe dengue: hemodynamic shock, severe bleeding, severe organ dysfunctions.** **#Dengue with warning signs: persistent vomiting, plasma leakage (pericardial effusion, ascites, pleural effusion), increased hematocrit for age and sex or 10% increase in basic hematocrit, postural hypotension or syncope, any spontaneous bleeding, severe prostration, severe abdominal pain, Hepatomegaly (> 2cm from rib cage)** ALT: Alanine aminotransferase; LSN: Upper limit of normality; GFR: Glomerular filtration rate, estimated by the 2021 The *Chronic Kidney disease epidemiology collaboration (CKD-EPI)* formula with the participant's serum creatinine value, gender, and age.	
Planned sample size	60 Participant in each arm of the study. 120 participants in total	
Duration planned study	2 years for the total duration of the trial and 28 days for individual patient engagement	
Intervention	**RESOMELAGON 100mg PO once daily** + standard treatment	
Control	Placebo + standard treatment	
Justification	a. To evaluate the safety of RESOMELAGON in patients infected with dengue. The drug has been studied in healthy individuals and patients with rheumatoid arthritis and COVID-19 with favorable safety parameters, but never in dengue. Therefore, safety data are needed for future phase 3 trials. b. To evaluate efficacy trends of RESOMELAGON. Dengue is a disease with inflammatory pathogenesis, but currently no drug has been proven to be effective. A drug that is capable of reducing the severity of the disease is needed for this infectious disease prevalent both in Brazil and in other developing countries.	
	Objective	**Endpoint**
Primary Safety	To evaluate the safety of RESOMELAGON in patients infected with dengue	Frequency of adverse events and comparison of hematological, biochemical, and virological parameters.
Primary Efficacy	Efficacy of RESOMELAGON in reducing the duration of disease	Time to disease resolution as defined by a composite outcome:

		a. Patient afebrile for 48 hours with or without antipyretic medications; b. Increasing platelet counts of >20% of the lowest count, or normal platelet counts if the patient did not have thrombocytopenia, after two consecutive measurements of the lowest platelet count c. Stabilized hematocrit or return to normal hematocrit for age and sex, after two consecutive measurements d. Absence of significant bleeding or vomiting for 48 hours.
Secondary Efficacy	Efficacy of RESOMELAGON in Reducing Disease Severity	Incidence of dengue with warning signs or severe dengue in the study patients
	Efficacy of RESOMELAGON in Reducing Plasma Extravasation	Evidence of pleural effusion OR evidence of ascites OR pericardial effusion OR incidence of hemoconcentration (hematocrit increase of >10% of inclusion or elevated for age and sex)
	Efficacy of RESOMELAGON in reducing dengue hospitalization or prolonged observation in the emergency department, as both represent a burden on health systems	Incidence of stay of more than 12 hours in the emergency department or hospitalization
Tertiary	To evaluate mechanisms that may explain the mechanism of action of RESOMELAGON in patients infected with dengue	Measurement of cytokines and chemokines and other parameters involved in inflammation and resolution, leukocyte tests for microvesicles, and other laboratory parameters considered potentially significant.

2. LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ADLs	Activities of Daily Living
REC	Research Ethics Committee
CRF	Case Report Form
CTCAE	Common terminology criteria for adverse events
DSMB	Data Safety and Monitoring Board
AE	Adverse Event
SAE	Serious Adverse Event
EDTA	Ethylenediaminetetraacetic acid
PPE	Personal protective equipment
IV	Intravenous
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IM	intramuscular
BMI	Body Mass Index
WHO	World Health Organization
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
SOP	Standard Operating Procedure
IMP	Investigational Medicinal Product
AR	Adverse reaction
SAR	Serious Adverse Reaction
RT-qPCR or qPCR	Real-time quantitative polymerase chain reaction
SARS-CoV2	Severe acute respiratory syndrome coronavirus 2
ICF	Informed Consent Form
eGFR	Estimated Glomerular Filtration Rate
TNF	Tumor necrosis factor
U&E	Urea and Electrolytes

3. BACKGROUND AND REASONING

The situation for the treatment of diseases of social determination caused by arboviruses, which in Brazil mainly includes Dengue, Chikungunya, Yellow Fever and Zika, is quite bleak. In fact, in the year 2024, we had the worst epidemic in the country's history, with more than 16 million cases and 5 thousand deaths. Although some prevention measures are beginning to be available for some arboviruses, there are no antivirals with a demonstrated clinical effect for these diseases. Experience with COVID-19 has clearly shown the beneficial effect of treatment with antivirals (e.g. Paxlovid) and anti-inflammatory drugs (eg. Glucocorticoids) in the evolution of the disease. In this project, we will focus on the attempt to develop a drug with the potential to change the natural history of dengue.

In the last two decades, Brazil has been facing constant epidemics of ***dengue fever*** with increasing numbers of cases of illness and death, with the largest epidemic in history occurring in 2024. Although there is already a vaccine approved a few years ago (DengVaxia) and another approved last year (2023 – Qdenga), the use of these vaccines is not yet widespread and they are not effective against all types of dengue. There are advances in the use of innovative vector control strategies (such as the use of mosquitoes with the Wolbachia bacterium), but these also do not have widespread application in our environment or even in the world. Finally, and of interest to the present study, (1) (2) (3) ***there are no antiviral drugs*** or with another mechanism of action approved for use in patients with Dengue. In fact, there are no drugs that prevent the evolution to the most severe conditions or that modify the course of the infection in those who have evolved to the most severe forms of the disease.

The inflammatory response is an articulated and complex reaction of our body to external and internal threats, with the aim of controlling them and accelerating the recovery of normal physiological functions. In the last 20 years, studies have emerged that have demonstrated that a protective, efficient, and non-chronic inflammatory response is composed of an initial phase, characterized by a predominance of pro-inflammatory mediators, followed by a resolution phase that ends the entire response with a return to a new state of homeostasis. Although in the past the inflammation resolution phase was considered a passive phenomenon (e.g., occurring due to the absence or catabolism of pro-inflammatory mediators), it is now fully appreciated that specific mediators, signaling pathways, and mechanisms should be used to promote the resolution phase of the inflammatory process.

When inflammation persists, it becomes "pathological" and, as such, contributes to the pathogenesis of the vast majority of chronic diseases that affect the general population, such as arthritis, cardiovascular diseases, diabetes, liver diseases, and degenerative pathologies of the central nervous system. The chronic inflammation phase is irreversible and causes deleterious effects on tissues and organs, affecting the body's overall homeostasis. Since the inflammation-resolution phase is an active process, chronic inflammation can result not only from an overload of pro-inflammatory mediators and mechanisms, but also from inadequate or insufficient involvement of problem-solving processes. This opens up a possibility for therapeutic innovations since almost all anti-inflammatory drugs, with the exception of corticosteroids, act on the inhibition of the inflammatory phase and not on pro-resolutive mechanisms (4) .

Although most Dengue virus infections are mild, about 5% of cases evolve with an exacerbated inflammatory response that progresses to endothelial injury and, thus, plasma leakage. Such a complication is known as severe dengue and can lead to hypovolemic shock, sometimes fatal. It is our hypothesis that mechanisms of regulation of inflammation resolution are altered in viral diseases such as dengue. Thus, the administration of pro-resolutive molecules could be useful to slow down or prevent the complication of dengue. In fact, the potential benefits of a pro-problem-solving treatment with Annexin A1 have already been described in a murine model in dengue, reducing thrombocytopenia, vascular permeability, and pro-inflammatory cytokines. Thus, we established a proof of concept that the administration of pro-resolutive molecules, which are also anti-inflammatory, are beneficial in the context of dengue. (5) (6,7) (8)

RESOMELAGON, a small-molecule drug, is a "biased" agonist of melanocortin receptors 1 and 3 (MCR1 and MCR3). In the last decade, it has been shown that the melanocortin system has several pro-resolutive effects. MCR agonists, primarily adrenocorticotropic hormone (ACTH) and αMSH, as well as synthetic analogues such as AP214 peptide, are anti-inflammatory and have organ-protective effects in various in vivo models. In addition, CMs have been shown to induce pro-resolutive effects such as phagocytosis and efferocytosis, which

gives a new aspect to CM-based therapy. RESOMELAGON presents advantageous pharmacology because it presents a biased activation of intracellular signaling pathways, by the phosphorylation of kinases regulated by extracellular signal (ERK 1/2) and intracellular calcium influx without causing cyclic AMP formation. At the functional level, RESOMELAGON modulated inflammatory responses in experimental animals and exerted pro-resolutive effects on immune cells, including promoting efferocytosis and inhibiting cytokine release. In more detail, the compound: 1) reduced the release of pro-inflammatory cytokines such as IL-1β and IL-6 from stimulated macrophages in an ex vivo setting; 2) increased the capacity of macrophages for phagocytosis and efferocytosis, which indicates a phenotypic shift from type 1 (M1) to a type 2 (M2), with this phenomenon playing a central role in the pro-resolutive effects of the compound; 3) reduced cytokine release (9) *in vivo*, both in LPS-induced models of systemic inflammatory stress and in models of peritonitis, and 4) promoted significant acceleration of resolution in a model of peritonitis when administered therapeutically up to 12 hours after disease induction. In addition, biased agonism against cAMP accumulation implied that RESOMELAGON did not activate melanin biosynthesis. The latter is potentially a significant advantage compared to the classical MCR (and ACTH) agonist, where the most undesirable side effect is the induction of skin pigmentation, after repeated dosing. (10)

3.1. Preclinical results of RESOMELAGON in Dengue

Using a preclinical model of dengue infection with A129 mice (deficient for the type I interferon receptor), we evaluated the impact of therapeutic treatment with RESOMELAGON on disease progression. Treatment was initiated 36 hours after infection with 2×10^4 PFU of DENV-2, administered subcutaneously at a dose of 20 mg/kg every 12 hours. Our results demonstrate that RESOMELAGON is able to significantly delay the appearance of clinical signs, such as weight loss, conjunctivitis, hair bristling and reduced locomotor activity, compared to the control groups (MOCK and vehicle). In addition, the treatment was effective in preventing thrombocytopenia, observed by maintaining platelet counts at levels significantly higher than those in the untreated groups. Additionally, biochemical analyses of the plasma revealed that RESOMELAGON promotes a significant reduction in the production of inflammatory mediators, such as MCPT-1 and CCL5, known markers of mast cell activation and inflammation, respectively. These anti-inflammatory effects were accompanied by the absence of significant impact on plasma viral titers, as demonstrated by the quantification of viable viruses by titration assay in Vero CCL cells81. These findings indicate that RESOMELAGON acts as a modulator of the inflammatory response in the preclinical model, contributing to the improvement of the clinical picture and the preservation of platelets without directly interfering with viral replication.

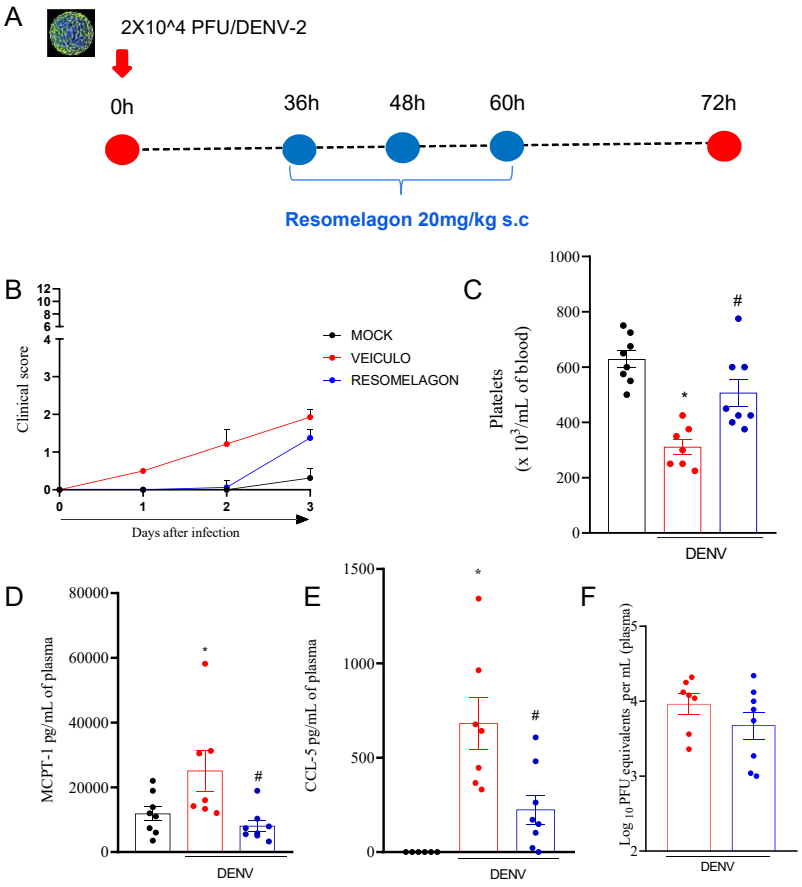


Figure 1: Treatment with RESOMELAGON **delays the onset of clinical signs, prevents thrombocytopenia, and reduces the production of inflammatory mediators, without impacting viral titers in plasma. A)** Experimental scheme: A129 mice (deficient for the type I interferon receptor) were infected with 2×10^4 DENV-2 PFU subcutaneously (intraplantar). Treatment with RESOMELAGON was initiated 36 hours after infection, at a dose of 20 mg/kg every 12 hours, subcutaneously. Euthanasia was performed 72 hours after infection for analysis. **B)** Clinical score evaluating weight loss, conjunctivitis, hair bristling and reduced movement. **C)** Platelet count expressed as platelets × 10^3/mL of blood. **(E)** Quantification of MCPT-1 and CCL5 in plasma by ELISA, with results expressed in pg/mL. **F)** Viable viral titers in plasma quantified by titration in permissive Vero CCL81 cells, expressed in Log10 PFU/mL of plasma. Statistical analysis by one-way ANOVA. *p<0.05 in relation to the MOCK group; #p<0.05 in relation to the vehicle group.

3.2 Summary of ascending dose trials

The compound was tested in a randomized, double-blind, placebo-controlled, single-ascending dose study with RESOMELAGON or placebo administered as an oral suspension. Seven male groups with healthy volunteers (48 males) and one female group with eight healthy postmenopausal females (defined by at least a two-year amenorrhea period and a follicle-stimulating hormone (FSH) level> 30 IU/L) received a single ascending dose with RESOMELAGON/placebo administered as an oral PSI suspension dissolved in SyrSpend ALKA under fasting conditions. The dose range tested was in men from 15 to 800 mg. The dose tested in women was 400 mg.

Pharmacokinetic (PK) results showed that in male participants in the dose range of 50 to 800 mg, RESOMELAGON was rapidly absorbed with a median T_{max} (time at which C_{max} is observed) between 1 and 4 hours. Inter-individual variability was low to moderate for Cmax (maximum plasma concentration) and AUCs (area under the curve) (<36%). The mean $t_{1/2}$ was close to 20 h (CV% <20%). The mean Vd/F (apparent volume of distribution) was between 556 and 784 L with CV% below 35%, and the mean CL/F (apparent total clearance) was between 20.26 and 31.26 L/h with CV% below 50%.

In female participants, following administration of 400 mg RESOMELAGON, C_{max} and AUCs were slightly increased compared to male participants. The apparent clearance was lower in the female population (17.26 L/h), and the apparent volume of distribution was similar. However, statistically, no conclusions can be made about the gender effect.

Twenty (20) participants reported a total of 38 Treatment-Emergent Adverse Events (TEAEs). All TEAEs were mild (27) or moderate (11) in severity. No serious adverse events were reported during the study. Twenty-two (22) of the 38 TEAEs were considered to be treatment-related in the study: 21 of these events occurred after administration of 200 to 800 mg of RESOMELAGON and one event occurred after administration of placebo.

The most frequent treatment-related TEAEs were gastrointestinal disturbances (19 events reported per fourteen (14) subjects): nausea (7 events), abdominal pain (5 events), vomiting (3 events), diarrhea (2 events), bloating (1 event), and dyspepsia (1 event). The frequency of these related TEAEs increased with dose. However, the amount of vehicle in the suspension increased with dose, leading to thicker consistency and worse taste of the study drug. This change in consistency and flavor of the suspension may be involved in this effect. Among TEAEs considered unrelated, headache was reported by seven (7) subjects. Other TEAEs were sporadic.

A total of four (4) male participants treated with active PSI had isolated increases in aminotransferases (no concomitant changes in alkaline phosphatases or bilirubin were reported). The increases, which amounted to up to 1.6 above the upper normal value of alanine transaminase (ALT), were observed in one (1) participant subject treated with 400 mg, two (2) participant subjects treated with 600 mg, and one (1) participant subject treated with 800 mg. No increases in aminotransferases above the upper normal range were observed in placebo-treated participants.

No RESOMELAGON-associated increases in QTcF (the Fridericia-corrected QT interval) nor changes in any other cardiac parameters were identified during the continuous 24-hour assessment of the 1000 HZ Holter ECG recording. No treatment-related changes in vital signs were observed.

Based on the safety assessment, it can be concluded that the maximum tolerated dose (MTD) has not been reached. At the maximum administered dose (MAD), the exposure obtained was more than 10x above what is expected to be the exposure level to achieve therapeutic efficacy. Therefore, it was decided not to continue to increase the dose above 800 mg.

3.2. Summary of repeated dose trials

The compound was tested in a randomized, double-blind, placebo-controlled, repeat-dose study with RESOMELAGON or placebo administered as an oral suspension administered once daily for 14 days. Three trials of 12 participants (9 active; 3 placebo) were administered with the same formulation used in part 1 of the study. Dose levels tested were 50 mg, 100 mg, or 200 mg with matching placebo.

Pharmacokinetic evaluation showed that **Cmax was observed between 1 and 2.5 hours post-dose**, regardless of the day of dosing. Steady state was reached on the 7th. At steady state, the C_{max} at the 50 mg dose level was about 180 ng/mL (group average), which is expected to be the exposure level required to induce anti-inflammatory efficacy. Dosing at the 100 mg dose level reached C_{max} around 400 ng/ml (group average) at steady state, i.e., a maximum increase of approximately 2-fold, compared to the 50 mg dose level. Dosing at the 200 mg dose level identified C_{max} at 900 ng/ml (group average) with the highest measured level of 1,300 ng/ml. Since the expected peak exposure required to induce therapeutic efficacy is expected to be around 170-180 ng/ml, exposure at 200 mg dose levels, based on C_{max}, is up to 5x and, for the subject with the highest exposure, more than 7 times the expected therapeutic value.

Fourteen (14) subjects reported a total of 35 Treatment-Emergent Adverse Events (TEAEs). All TEAEs were mild (27) or moderate (8) in severity. No serious adverse events were reported during the study. Four (4) of the 35 TEAEs were considered possibly related to study treatment. All four of these gastrointestinal disturbances-related events were observed following administration of the investigational drug (two (2) in the same subject, at a dose of 50 mg (one episode of diarrhea and one episode of abdominal cramp, two (2) in the same subject

at the dose level of 200 mg (one episode of nausea and one episode of vomiting). Among TEAEs considered unrelated, nine (9) participants reported headache, seven (7) treated with the investigational drug, and two (2) treated with placebo. Other TEAEs were sporadic.

Some mean changes and individual abnormalities were observed in laboratory parameters, vital signs, and ECG parameters. Most of these changes and abnormalities were limited and considered not clinically significant. QTcF assessment of a continuous 1000 Hz Holter ECG recording performed on day 14 and compared to day 1 (baseline) is still ongoing based on exposure/response statistical analysis. No clinically significant individual QTcF value was observed at any time in this study on the standard 12-lead safety ECG.

A total of five (5) participants, all of whom were included in Cohort 3 (200 mg), three (3) active-treated and two (2) placebo-treated had isolated increases in aminotransferases (no concomitant changes in alkaline phosphatases or bilirubin were reported). The increases were more pronounced in the active-treated subjects, where the increase reached up to 3.6x and 2.9x above the upper normal value of ALT. After completion of the study, all values returned to normal.

Based on the safety assessment, it can be concluded that the BAT was not achieved. At the maximum administered dose (MAD), exposure was 5x above what is expected to be the exposure level for therapeutic efficacy. Therefore, it was decided not to continue to escalate the dose above 200 mg per day.

Consequently, RESOMELAGON has an excellent safety profile, allowing it to be administered to patients at doses of 50 mg or 100 mg for four weeks. Additional information about RESOMELAGON, including its physicochemical properties and the results of non-clinical in vitro and in vivo pharmacology, pharmacokinetics, and toxicology studies, is presented in the Investigator's Brochure.

3.3. Phase 2 clinical trial in COVID-19

A double-blind, placebo-controlled phase 2 clinical trial was conducted with 60 participants to evaluate the safety and efficacy of RESOMELAGON (100 mg/day) for the treatment of COVID-19, an infectious disease also with a significant pro-inflammatory pathogenesis. In this study, RESOMELAGON was shown to be safe, with no significant increase in adverse events compared to the control group, with only a tendency to the occurrence of gastrointestinal events. There were also no significant laboratory abnormalities. Although preliminary, a 2-day reduction in time to respiratory recovery was also demonstrated, with a time of 6 (IQR 4.12) days in the placebo group and 4 (IQR 3.7) in the treatment group (p=0.017). A reduction in length of hospital stay was also observed from 7 (IQR 5, 14) in the placebo group to 6 (IQR 4.8) in the RESOMELAGON group (p=0.038). (11)

3.4. Phase 2a Clinical Study in 105 Patients With RA

In the Phase 2a study, RESOMELAGON was administered as a once-daily oral suspension for four weeks as an add-on to methotrexate (MTX) in patients with active rheumatoid arthritis (RA) who were MTX-naïve and had high disease activity (Clinical Disease Activity Index (CDAI) >22 at baseline). The study included 105 patients randomized in a 2:1 ratio to the active drug versus placebo, in a two-arm design.

- **Part A**: Two groups received the active drug (50 mg and 100 mg) versus placebo.
- **Part B**: Participants received the active drug/placebo according to one of three study regimens:

1. 50 mg active medicine/placebo
2. 100 mg active medicine/placebo
3. Or 50 mg or 100 mg of active drug versus placebo.

None of the participants in the active treatment groups experienced abnormal or clinically significant changes in vital signs during study participation. The incidence of adverse events (AEs) was slightly higher in the RESOMELAGON groups compared to placebo, primarily due to nausea, constipation and headache. AEs in the

RESOMELAGON-treated groups were predominantly mild, and no severe cases were reported. No signs of immunosuppression were identified in any of the three treatment groups. There were no significant differences in liver enzyme abnormalities between the groups receiving 50 mg RESOMELAGON and placebo. No increase in liver enzymes was observed in participants treated with 100 mg RESOMELAGON. The 12-lead ECG assessment performed at each visit did not identify clinically significant abnormal changes. In addition, based on the assessment of RR interval, PR interval, QRS duration, QT interval, and QTcF interval, no treatment-related changes were identified in any of the three groups. There were no reports of serious adverse events (SAEs) or deaths during the study.

3.5. Phase 2a Clinical Study in 122 Patients With RA

This study was a multicenter, randomized, double-blind, placebo-controlled, and divided into two parts, with daily administration of RESOMELAGON. The study population consisted of participants with highly active RA (CDAI >22) and inadequate response to treatment with DMARDs (DMARD-IR) but without the need for elevated CRP at baseline. The first part of the study (Part A) was aimed at the preliminary evaluation of the safety and efficacy of the 4-week treatment with 60 mg, 80 mg, and 100 mg of RESOMELAGON once daily, with approximately 30 participants per group (1:1:1:1). A total of 122 participants were enrolled and 113 (92.6%) completed the study. The results of Part A indicated that RESOMELAGON was safe and well tolerated.

3.6. Phase 2b Clinical Study in 127 Patients With RA

This was a multicenter, randomized, double-blind, placebo-controlled, 12-week study with daily administration of RESOMELAGON. The study population consisted of newly diagnosed patients with highly active RA (CDAI >22), without the need for elevated CRP at baseline. The primary objective was to evaluate the safety and efficacy of 12-week treatment with 100 mg RESOMELAGON once daily. A total of 127 participants were enrolled in the study, and 114 (89.9%) completed the study.

Overall, RESOMELAGON was well tolerated; Similar rates of treatment-emergent adverse events (EAAEs) were observed between treatment groups (44.4% and 42.2%). Overall, treatment-related EAETs were reported in 11.1% in the RESOMELAGON group versus 6.3% in the placebo group. Treatment-related EAETs included upper respiratory tract infections (6.3% vs 6.3%), upper abdominal pain (6.3% vs 3.1%), nausea (6.3% vs 3.1%), headache (0% vs 9.4%), and vomiting (6.3% vs 0%) (incidence rates listed for RESOMELAGON vs placebo, respectively).

No clinically significant abnormal observations were noted in the changes from baseline in the mean, median, or maximum/trough values in safety parameters (chemistry/hematology), vital signs, physical examinations, or ECG. Some changes from normal to abnormal were observed in categorical laboratory parameters and some individual values were reported as EAETs (none of these were considered drug-related).

Consequently, the study showed no sign of RESOMELAGON-associated effects on liver or kidney function, nor did it indicate RESOMELAGON-induced immune system suppression.

4. OBJECTIVES AND OUTCOMES

	Objectives	Endpoints	Time of endpoint assessment
Primary safety	To evaluate the safety of RESOMELAGON in patients infected with dengue	Frequency of adverse events and comparison of hematological, biochemical, and virological parameters.	Days 0-28
Primary efficacy	Efficacy of RESOMELAGON in reducing the duration of disease	Time to disease resolution as defined by a composite outcome:	Days 0-14

		a. Patient afebrile for 48 hours with or without antipyretic medications; b. Increasing platelet counts of >20% of the lowest count, or normal platelet counts if the patient did not have thrombocytopenia, after two consecutive measurements of the lowest platelet count c. Stabilized hematocrit or return to normal hematocrit for age and sex, after two consecutive measurements d. Absence of significant bleeding or vomiting for 48 hours.	
Secondary efficacy	Efficacy of RESOMELAGON in Reducing Disease Severity	Incidence of dengue with warning signs or severe dengue in the study patients	Days 0-14
	Efficacy of RESOMELAGON in Reducing Plasma Extravasation	Evidence of pleural effusion OR evidence of ascites OR pericardial effusion OR incidence of hemoconcentration (hematocrit increase of >10% of inclusion or elevated for age and sex)	Days 0-14
	Efficacy of RESOMELAGON in reducing dengue hospitalization or prolonged observation in the emergency department, as both represent a burden on health systems	Incidence of stay of more than 12 hours in the emergency department or hospitalization	Days 0-14
Tertiary	To evaluate mechanisms that may explain the mechanism of action of RESOMELAGON in patients infected with dengue	Measurement of cytokines and chemokines and other parameters involved in inflammation and resolution, leukocyte tests for microvesicles, and other laboratory parameters considered potentially significant.	Days 0-14

5. STUDY SITE

The study will be conducted at the clinical research unit of the CT Advanced and Innovative Therapies at UFMG (Federal University of Minas Gerais. Participants may be recruited from health centers or Emergency Care Units and taken to the study site for follow-up procedures.

6. STUDY DESIGN

This study is a randomized, double-blind, placebo-controlled, multicenter, repeat-dose clinical trial of RESOMELAGON in patients with acute dengue infection.

6.1. Intervention

- RESOMELAGON 100mg orally once daily
- Placebo

Investigational product (PSI) will be administered for 5 days. The first dose will be administered at the time of inclusion and then daily in the morning fasted (1 hour before breakfast).

Note: All study participants will also receive supportive treatment from the local health system; for example, antipyretics and analgesics, according to the judgment of the attending physician.

7. PATIENT IDENTIFICATION AND RECRUITMENT

7.1. Study participants

Adult patients with symptomatic acute arbovirus syndrome with less than 72 hours of symptoms and DENV infection confirmed by antigen or polymerase chain reaction.

7.2. Inclusion criteria

1. **Participants aged 18 to 65 years;**
2. **Participant is able to understand study procedures and provide informed consent;**
3. **Presents more than 36 and less than 84 hours after the onset of symptoms;**
4. **Presents with symptoms consistent with dengue infection with a positive antigen test or positive PCR test. Dengue is suspected by the report of fever and at least 2 other signs or symptoms of the disease, including myalgia, arthralgia, headache, or conjunctivitis).**

7.3 Exclusion criteria

1. **Has any comorbidity that is perceived as decompensated by the investigator;**
2. **The following significant laboratory abnormalities discovered at screening would lead to participant discontinuation:**
    - **Hemoglobin <10g/dl**
    - **Platelet count <50,000/Ul**
    - **ALT> 3x ULN**
    - **Total bilirubin> 1.5 x ULN**
    - **GFR <60mls/min/1.73m2**
3. **Contraindications or known hypersensitivity to RESOMELAGON ;**
4. **Presents as dengue with warning signs# or severe dengue* at inclusion;**
5. **Currently participating in another clinical drug trial;**
6. **Clinical evidence of another infection that may explain the current symptoms;**
7. **Pregnant women or women actively trying to achieve pregnancy.**

***Severe dengue: hemodynamic shock, severe bleeding, severe organ dysfunctions.**

#Dengue with warning signs: persistent vomiting, plasma leakage (pericardial effusion, ascites, pleural effusion), increased hematocrit for age and sex or 10% increase in basic hematocrit, postural hypotension or syncope, any spontaneous bleeding, severe prostration, severe abdominal pain, Hepatomegaly (> 2cm from rib cage)

ALT: Alanine aminotransferase; LSN: Upper limit of normality; GFR: Glomerular filtration rate, estimated by the 2021 The *Chronic Kidney disease epidemiology collaboration (CKD-EPI)* formula with the participant's serum creatinine value, gender, and age.

8. INCLUSION AND PROCEDURES IN THE STUDY

Patients with a suspected diagnosis of acute arbovirus coming from basic units or emergency rooms will be informed of the study and the possibility of performing the diagnostic test. Suspicion of dengue is given by the report of fever and at least 2 other signs or symptoms of the disease, including myalgia, arthralgia, headache or conjunctivitis. For patients who can be included, the NS1 rapid test and RT-PCR test for the diagnosis of dengue will be offered, if these tests have not yet been performed. A rapid test for COVID-19 may be done to exclude individuals with this disease, if the attending physician deems it necessary. The RT-PCR test is expected to be available within 24 hours of obtaining the blood sample. Recruitment can occur at any time up to 84 hours after symptom onset, but inclusion and randomization should occur between 36 and 84 hours after the reported onset of symptoms. Patients with a positive RT-PCR or NS1 test for dengue will be enrolled in the study after written informed consent.

After inclusion, all recruited patients will be visited at their home or place of residence or will be seen at the referral Clinical Research Unit. All employees will wear appropriate personal protective equipment (PPE), in accordance with local health system guidelines. For home visits, a trained staff member will take a blood sample.

During the baseline visit, participants will be asked about their symptoms and their medical history. The patient will undergo a physical examination and blood draw for routine hematology and biochemistry and other initial investigations. After inclusion in the study, the patient will be randomized to one of the study arms.

A blood sample will be obtained using a standard operating procedure (SOP) prior to receiving the first dose of study drug. The first dose of RESOMELAGON or placebo will be administered under observation at the clinical research unit. Subsequent doses will be self-administered by the participants, with a time recorded in the diary and checked by the study team during the visits. There will also be blood collections on the days determined in the schedule of activities.

8.1. Virological sampling

Serial blood samples will be collected according to the sample schedule by study personnel trained according to a standard operating procedure (SOP) established for this purpose. Daily sampling will be performed by study personnel at the participant's residence or at the study center. Samples will be kept cooled and subsequently stored at -80°C as detailed in the SOP. Transport times will be recorded, as well as the time for the plasma to be frozen at -80°C. Viral genomes from blood samples will be quantified by RT-qPCR. Patient measurements over time will be used to estimate the rate of viral clearance.

All dengue-positive samples will be typed to determine the effect of the subtype on viral load and response to treatment. This will be done using whole-genome sequencing technology or specific PCRs to determine subvariants.

8.2. Recruitment

Potential patients with symptoms suggestive of acute arboviral disease will be invited to participate in the study. Suspicion of dengue is given by the report of fever and at least 2 other signs or symptoms of the disease, including myalgia, arthralgia, headache or conjunctivitis. Folders will be distributed in basic health units and/or in urgent care centers informing about the study. We will also promote it through social media. Participants can contact the research group for more information about the study. All procedures and follow-ups of the participants will take place in the reference Clinical Research Units where the study is taking place. If the patient has a compatible clinical condition, but less than 36 hours after the onset of symptoms, the patient can be recruited for later inclusion after 36 hours of symptoms.

8.3. Eligibility screening and assessment

Eligibility assessment will take place at the time of screening. If, based on the inclusion and exclusion criteria, the patient is eligible and willing to complete the full study, they will be enrolled in the study after obtaining informed consent.

8.4. Informed consent

Written versions of the participant's information and the informed consent form (ICF) will be presented to patients detailing the exact nature of the study; what it will involve for the patient; the implications and constraints of the protocol; and the known side effects of the drugs under evaluation and any risks involved in their participation. It will be clearly stated that the patient is free to withdraw from the study at any time for any reason, without prejudice to future care and without obligation to provide the reason for withdrawal.

The patient will have all the time necessary to consider the information and will have the opportunity to question the investigator or other independent parties to decide whether they will participate.

Informed consent will then be documented in two copies signed and dated by the patient and the person who submitted and obtained the informed consent. The person who obtained consent must be appropriately qualified and experienced and have been authorized to do so by the site's PI. One copy of the informed consent signed and dated by the participant and the researcher who obtained the consent will be given to the patient and the other copy will be kept on file at the study site.

8.5. Baseline assessments (See Appendix 1 for detailed schedule of activities)

A physical examination, vital signs, oxygen saturation, symptom review, and basic demographics will be recorded. If there is no reason for exclusion for clinical reasons, the participant will be included, randomized and will receive the first dose of the drug for which they are drawn. A total of 40 ml of venous blood will be collected at this time for the evaluations related specifically to the study. The participant may be discontinued prior to the next dose if there are laboratory abnormalities that result in contraindication to IMP. If a complete blood count and biochemistry were obtained within 36 hours prior to study inclusion, the results can be used to determine if there is a contraindication to your participation in the study.

Laboratory tests at baseline:

- Blood sample for
    - Blood count and differential count (Reference laboratory of the participating Clinical Research Unit);
    - Biochemistry (urea, creatinine, liver function tests and albumin) (Reference laboratory of the participating Clinical Research Unit);
    - Cytokines, IL-6, soluble TNR1 and TNF2 receptors, and the chemokines CXCL8 and CCL2 (Central analysis at CT Terapias UFMG);
    - RT-PCR and detection of the NS1 virus (Central analysis at CT Terapias UFMG);
    - Buffy-Coat will be used to study the response of circulating leukocytes to infection.
    - Dengue serology (IgG, antibody responses) to assess previous exposure to dengue (Central analysis at CT Terapias UFMG);

Note: Cytokine evaluations, including IL-6, soluble TNR1 and TNF2 receptors, and the chemokines CXCL8 and CCL2 are important markers found in the plasma and serum of dengue patients and mark the intensity of the inflammatory response. For these analyses we will use commercial Elisa kits (DuoSet from RND systems) following the manufacturer's instructions

8.6. Study days

8.6.1. Day 0

After inclusion, the participant will be randomized to one of the study arms (RESOMELAGON or placebo) using envelopes previously drawn and defined by the study statistician. Randomization rates will be uniform for participants (1:1). Only the designated study team (unblinded staff) will be allowed to access the envelopes for patient allocation.

For participants randomized to either RESOMELAGON or placebo, the first dose of treatment (hour 0) will be supervised by the study team. The participant will then be observed for a minimum of 1 hour in the Clinical Research Unit before being released home.

8.6.2. Days 1 to 4, Day 10 and Day 14*

During these days, a sample of up to 40 ml of venous blood will be obtained, if there is no contraindication. The following variables will be evaluated daily:

- Treatment evaluation (the administration of the medications taken must have the time recorded by the participant (days 1-4).
- Axillary temperature recorded twice daily by study staff or participant (information collected in a diary and reviewed daily by study staff) only up to day 7.
- The study team completes a brief checklist of symptoms every day
- Evaluation of Adverse Events (AEs) and Concomitant Medications (CM).
- Blood sample collected for biochemical laboratory tests (urea, creatinine and liver function) and complete blood count according to schedule (appendix 1).
- Blood samples collected for evaluation of cytokine levels, leukocyte testing (buffy-coat), RT-PCR and NS1, according to schedule (appendix 1).
- Medical evaluation on D7 with diary collection.

***See Appendix 1 for a detailed schedule of activities.

8.6.3. Day 28 (1 month) assessment

The following variables will be assessed on days 28 (1 month ± 4 days) by telephone contact:

- The study team completes a brief symptom checklist;
- The participant will be asked about AEs and CMs;
- Participation in the study will be completed.

8.7. Management of patients who become ill

During the study, although this is unlikely as we are recruiting patients at low risk of developing more severe disease, the patient may become clinically worse or may develop a new intercurrent illness or potentially a side effect related to the study medication. All patients who develop difficulty in activities of daily living or worsening of their symptoms will be referred to the study physicians for a clinical evaluation. The clinical deterioration in this study may be due to several factors, including progression of dengue disease, development of complications such as shock, adverse drug reaction, among others. Thus, a thorough clinical evaluation will be performed by the study physician. Based on this assessment, they may be referred to an emergency care facility, re-examined the next day, or asked to update the mobile phone research team frequently on their well-being.

If a patient is referred to an emergency care unit, a clinical evaluation will be made by the attending physician and a diagnosis will be made. The decision to discontinue study drug will depend on this diagnosis and will be made between the site team and the study PI.

Physicians in the emergency care unit will have responsibility for patient care, but the research team will continue to track patients' progress in the hospital. Treatment for dengue fever severe enough to warrant hospitalization will follow national guidelines.

Blood samples will be tested for complete blood count and biochemistry (Urea, creatinine, and liver function tests) to assess for drug-related adverse effects or abnormalities requiring a possible dose adjustment or interruption of therapeutic or clinical intervention. These tests are also important for the clinical follow-up of the study participant. The responsibility for evaluating and acting on the results of the blood tests will belong to the study team. They will use their clinical judgment in participant assessment, additional testing, and further referral. At any time during the study, extra laboratory tests may be performed on the participant, if clinically indicated by the research team, based on previous laboratory symptoms or abnormalities.

8.8. Sample Handling and Retention

Samples will be transferred to designated testing facilities where they will undergo testing in accordance with best practices of laboratory measures and safety procedures.

Blood samples for virus detection will be processed using quantitative real-time quantitative polymerase chain reaction (qPCR) validated to detect dengue, according to the study's SOP. The kit to be used is a commercially available kit (Biomol ZDC kit) distributed by IBMP (Institute of Molecular Biology of Paraná, FIOCRUZ). Samples will be retained in accordance with local ethical regulations and approvals. Patients who consent may rescind their consent until the study is completed. To do so, participants must make a written request, sign and date. Unless requested, samples collected prior to the withdrawal date will be retained for study analysis.

Some of the remaining blood samples will be stored and can be used for further studies in the field of susceptibility or response to dengue infection and treatments. Blood samples will be labeled with a unique number and initials, but not with the patient's name. Any additional testing beyond that indicated in this study will be tested only after permission from the ethics committee and applying new ICF if deemed necessary.

9. DISCONTINUATION/WITHDRAWAL OF PATIENTS FROM THE STUDY

Participants may choose to discontinue treatment and/or study assessments, but may remain under study follow-up. Participants can also withdraw their consent, which means they want to withdraw from the study altogether. In the event of withdrawal of treatment and active follow-up, the following options for a staggered withdrawal from the study would apply

a) Patients withdraw from the study but allow data and samples obtained up to the point of withdrawal to be retained for use in study analysis. No further data or samples would be collected after withdrawal,

b) Patients withdraw from active follow-up and further communication, but allow study staff to continue accessing their medical records and any relevant hospital data that is recorded as part of the routine standard of care; i.e. CT scan, blood results and disease progression data, etc.
c) Patients withdraw, but do not allow the data and samples collected to be used.

In addition, the Investigator may **discontinue a participant from investigational treatment** at any time if the Investigator deems it necessary for any reason, including but not limited to:

- Pregnancy;
- Ineligibility (arising during the study or retrospectively, having been neglected at screening), this criterion will be evaluated on a case-by-case basis by the investigator, since participants who become ill will continue to be followed, for example;
- Significant protocol deviation;
- Significant non-compliance with the treatment regimen or study requirements;
- An adverse event that requires discontinuation of study drug or results in the inability to continue to comply with study procedures;
- Disease progression that requires discontinuation of study medication or results in the inability to continue to comply with study procedures.

The reason for discontinuation and/or withdrawal will be recorded on the Case Report Form. The participant will be followed by the clinical team of the study until the final outcome of the reason for their discontinuation, both due to illness and/or pregnancy.

Participant qPCR data taken from the study will still be analyzed if at least three distinct time points are available for the estimation of a clearance slope. The sample size is adaptable, so there is no need to replace withdrawn patients.

Consenting participants may rescind their consent until completion of the study. Unless requested, data and samples collected prior to the withdrawal date will be retained in the study database and analysis.

9.1. Definition of End of Study

The end of the study is the date of the last enrolled patient's 28-day follow-up visit.

10. SECURITY REPORT

9.1. Definitions

Adverse Event (AE)	Any untoward medical occurrence in a patient to whom the investigational product has been administered, including occurrences that are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An undesirable and unintended response in a patient to an investigational drug that is related to any dose administered to that patient. The phrase "response to an investigational drug" means that a causal relationship between an investigational drug and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by the qualified medical professional to have a reasonable suspicion of a causal relationship to the study drug qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: ● results in death; ● it is life-threatening; ● requires hospitalization or prolongation of existing hospitalization; ● results in persistent or significant disability/incapacity; ● It consists of a congenital anomaly or birth defect. Other 'major medical events' may also be considered serious if they endanger the patient or require an intervention to prevent one of the above consequences.

	NOTE: The term "life-threatening" in the definition of "severe" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically could have caused death if it had been more serious. Hospitalization is defined as a formal, unplanned hospitalization, even if hospitalization is a precautionary measure for continued observation. The patient must be hospitalized for at least one night; A short stay of several hours to receive treatment is not considered hospitalization. If a patient is admitted overnight or longer for social/economic or isolation reasons and is clinically stable, this does not constitute an SAE. Other examples of visits to a hospital facility that are not considered hospitalization are: emergency department visits, outpatient surgery, pre-planned or elective procedures for a pre-existing condition (provided that this condition has not worsened during experimental or anticipated treatment due to worsening symptoms) and for the purpose of this study, being hospitalized for Influenza isolation.
Serious Adverse Reaction (SAR)	An adverse event that is serious and, in the opinion of the reporting investigator, is believed to be reasonably likely to be due to one of the study treatments, based on the information provided.

NB: to avoid confusion or misunderstanding about the difference between the terms SAE and grade 3 AE "severe", the following note of clarification is provided: "Severe" is often used to describe the intensity of a specific event, which may be of relatively minor medical importance. EAG is the regulatory definition provided above.

Any pregnancy that occurs during the clinical trial and the outcome of the pregnancy must be recorded and followed for congenital abnormalities or birth defects, in which case it would fall under the definition of AGA.

10.2. Causality

The relationship of each adverse event to the study drug must be determined by a medically qualified individual according to the following definitions:

Definitely related:	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Probably related:	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.
Possibly related:	There is some evidence to suggest a causal relationship (e.g., because the event occurs within a reasonable amount of time after study drug administration). However, the influence of other factors may have contributed to the event (e.g., the patient's clinical condition, other concomitant treatments).
Probably unrelated:	There is little evidence to suggest the existence of a causal relationship (e.g., the event did not occur within a reasonable time after administration of the study drug) or other reasonable explanation for the event (e.g., the patient's clinical condition, other concomitant treatment).
Unrelated:	There is no evidence of any causal relationship.

10.3. Procedures for recording adverse events

A symptom checklist will be performed daily between D2 and D7, on D10 and D14 with a final follow-up assessment on D28 to assist in the identification of adverse events. The severity of adverse events will be assessed following the Common Terminology Criteria for Adverse Events (CTCAE) v5.0:

1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening, 5 = fatal.

AEs occurring in participants since study inclusion and during study participation that are observed by the investigator or reported by the patient with intensity grade 3 (severe) or higher will be recorded in the CRF, whether assigned to the study drug.

The following information will be recorded: description, start date and end date, severity, assessment of relationship to study drug, other suspected drug or device, and action taken. Follow-up information should be provided as needed.

AEs deemed to be related to the study drug, as judged by a qualified medical investigator, will be followed until resolution or the event is deemed stable. All these cases will be duly reported and discussed with the company that produces the IMP.

It will be up to the investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require removal of the patient from treatment. A patient may also voluntarily withdraw from treatment due to what they perceive as an intolerable AE. If any of these occur, the patient should undergo a final study evaluation and receive appropriate care under medical supervision until symptoms disappear or the condition becomes stable. Non-clinically significant abnormalities will not be graded or recorded in CRF.

9.4. Classification of laboratory abnormalities

Abnormal laboratory findings detected during the study or present at baseline and worsened after ingestion of study medication will be reported as AEs or SAEs. If they are found to be abnormal, the values will be graded according to CTCAE v5.0. AEs of severity grade 3 or higher or EAGs will be recorded in the CRF and will be followed up to grade 2 or lower, returned to baseline, or considered permanent. For laboratory results that are not available in CTCAE v5.0, the site investigator must determine whether or not the laboratory abnormality is clinically significant. If the site investigator believes that a laboratory abnormality is clinically significant, it should be reported as an adverse event and a grade should be identified as best as possible. Non-clinically significant abnormalities will not be graded or recorded in CRF.

It is important to emphasize that the grade determined in CTCAE v5.0 should not be confused with the investigator's assessment of whether the AE is a serious adverse event. A relatively low-grade laboratory abnormality may, for a given participant, determine hospitalization or be life-threatening, and relatively high-grade abnormalities may in certain circumstances not have major clinical significance and, therefore, have no formal SAE criteria. The definition of SAE should be made by the investigator considering the circumstance of the event.

9.5. Serious adverse event reporting procedures

All SAEs detected by the site investigator are to be reported to the DSMB within 24 hours of becoming aware of the SAE.

Further follow-up reports should be submitted, if necessary, until the SAE is resolved, is considered stable/permanent, or results in death. A final status must be determined for any ongoing SAEs at the end date of the study.

The site PI must also report the SAEs to the local ethics committee and regulatory authority in accordance with local requirements.

9.6. Data Security Monitoring Board (DSMB)

An independent Data Security and Monitoring Board (DSMB) will be created, composed of qualified volunteers with the necessary knowledge of clinical trials. The DSMB will receive summary reports of the study as defined by the bylaws or by ad hoc request, prior to each meeting. All data reviewed by the DSMB will be strictly confidential. A DSMB charter will outline its responsibilities and how it will operate.

An interim report will be prepared by the Statistician for the pre-specified interim analysis. In case of safety issues, additional information or formal interim reviews may be requested by the DSMB.

The DSMB will formally meet at the following times:

- after the first 30 patients were enrolled in the study
- At additional time points as indicated by the DSMB after its review if deemed necessary

All DSMB recommendations will be communicated. The PI will be responsible for submitting the DSMB summary reports in writing with the applicable recommendations to the local ethics committee and other applicable groups.

11. STATISTICS AND ANALYTICS

11.1. Sample size estimation and randomization

The main objective of the present study is to characterize the safety and efficacy of RESOMELAGON versus the placebo arm in patients with dengue, especially in reducing the duration of the disease.

The sample size estimate was calculated using a power analysis (*Power Analysis*) using data from the literature regarding the mean time until resolution of symptoms and laboratory abnormalities caused by the disease (12) : Time to resolution of fever in the placebo group of 136 h and variation of approximately 20% (5.6±1.1 days), time to resolution of thrombocytopenia (6.5±1.4) and hemoconcentration (7.0±1.7). Considering that hemoconcentration is a less frequent event, the sample size can be estimated by evaluating only the resolution of fever and thrombocytopenia. Also considering an effect size of 1 day in reducing the duration of the disease, power of 80%, alpha of 5% and loss of 10%, we arrived at a sample size of 120 (60 who will receive RESOMELAGON and 60 who will receive placebo).

The randomization of participants will utilize a centralized system in which only authorized persons will have access to the application for allocating patients to the placebo or RESOMELAGON group. The database will be password protected. All randomization activities will be tracked and assigned a timestamp along with the patient's anonymized study code, age, and gender.

10.2. Overview of statistical analysis

To evaluate the efficacy of the administration of Resomelagon on the primary outcome (time to resolution of the disease) of the patients included in the study, negative poisson or binomial models will be adjusted considering the existence of right-hand censorship and the dispersion of the data.

For the safety analyses and secondary endpoints, the results will be tabulated by comparing the treatment with the placebo group. Whole or continuous variables will be expressed as mean and standard deviation or median and interquartile range. Categorical variables will be presented with absolute and relative frequencies. Normality of variables will be tested with the Shapiro-Wilk test while equality of variance will be analyzed with the F or Bartlett test. Mean or median differences will be evaluated by Wilcoxon, Kruskal-Wallis tests, T-test or analysis of variance considering the number of groups and assumptions of each test. Associations between categorical variables will be tested by Fisher's exact test or Pearson's chi-square test. Pearson's or Spearman's correlation coefficients will be estimated to explore the existence of linear association between continuous or integer variables. Generalized linear and linear models will be used for multivariate structures and data with temporal or spatial relationships will be explored with random effects models or generalized estimation equations. Estimates of time to occurrence of an event will be made using non-parametric Cox models or equivalent. The significance level will be 5%.

12. DATA MANAGEMENT

12.1. Access to data

Direct access will be granted to authorized representatives, local ethics committees and regulatory authorities, and any host institution for monitoring and/or auditing of the study to ensure compliance with regulations. Outcome data and treatment assignment data will be made available for real-time analysis.

12.2. Data Processing and Record Keeping

Clinical study data will be recorded in CRFs developed for the study and entered into a password-protected database by the local study PI, nurse researcher, or designee. The study database will be built on RedCap, a clinical data management system in compliance with ICH GCP and FDA regulation, title 21 of the *Code of Federal Regulations* Part 11. It will be hosted on a secure server with restricted access. The study

database will include internal quality checks to identify data that appears inconsistent, incomplete, or inaccurate.

Steps will be taken to ensure that potentially harmful information is not disclosed to patients. Paper records (e.g., patient identification information for follow-up purposes, screening records, and signed ICFs) will be kept in locked cabinets; Electronic data will only be accessible to personnel with user accounts and passwords. The database contains an audit trail that keeps track of changes to the user's data and activity in the database. All electronic data will be stored on secure servers backed up daily, with off-site storage weekly.

On-site patient records will, taking into account the capacity of the sites, be stored in binders in a secure room with limited access. Records will be retained for at least five years after completion of the study or in accordance with local site regulation. The study database will be maintained indefinitely.

13. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with relevant regulations and standard operating procedures.

The study will be conducted in accordance with this protocol, the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP), and any applicable regulatory requirements. Monitoring will be conducted locally and supervised with the assistance of the MORU *Clinical Trials Support Group* (CTSG) according to a pre-specified risk-based monitoring plan to ensure compliance with the study protocol and applicable guidelines and regulations. Biological samples will be processed and stored according to the Research Center's SOPs.

Data validation will be carried out to identify errors or discrepancies and thus ensure the integrity, validity and accuracy of the data.

14. ETHICAL AND REGULATORY CONSIDERATIONS

This study will be conducted in patients who are unlikely to progress to severe disease. The investigated drugs may benefit the patient; that is, they shorten the duration of symptoms or decrease their severity, but are unlikely to have a significant adverse effect on the patient's illness and subsequent health. The funds will be set aside to cover hospital costs in the unlikely event of an adverse drug reaction. All assistance will be provided to the participant in case there is any health problem resulting from their participation in the study for as long as necessary.

Women who are pregnant, actively trying to conceive, or breastfeeding will not be able to participate in this study as it is not known whether any of the treatments tested will have additional benefits that outweigh any risks associated with pregnancy/breastfeeding. In case of pregnancy after inclusion in the study, the participant will be discontinued and followed until the pregnancy outcome.

14.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the current revision of the Declaration of Helsinki.

14.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and Good Clinical Practice (ICH GCP).

14.3. Approvals

The protocol, informed consent form, participant information sheet, and any proposed publicity materials will be submitted to local ethics committees for written approval. The Investigator will submit and, where necessary, obtain approval from the above parties for all changes to the original approved documents.

14.4. Patient confidentiality

The study team will ensure that the anonymity of patients is maintained. Patients will only be identified by a patient ID number in all study documents and in any electronic database. All documents will be stored securely and accessible only by study staff and authorized personnel. The study will comply with the General

Data Protection Law (LGPD), which requires that personal data not be kept as identifiable data for longer than necessary for the purposes in question.

14.5. Costs

Patients and their companions will have their expenses, such as food and transportation resulting from their participation in the study, reimbursed by the study, in accordance with current ethical regulations.

14.6. Risk

Resomelagon is well tolerated and safe, and has been tested in normal individuals, patients with rheumatoid arthritis, and patients with moderate COVID-19. The most common side symptoms include those associated with the gastrointestinal tract, including nausea, vomiting, and diarrhea, and mostly of low intensity. These are usually mild and resolve without the need for medical intervention. Risks associated with drawing blood during the study include local discomfort, occasional bleeding or bruising at the needle puncture site, and, very rarely, infection.

Regarding pregnancy, Resomelagon should not be used during pregnancy and its use is not recommended for women who may become pregnant and who are not using effective contraceptive methods. Pregnancy tests will be performed on all participants who may become pregnant prior to the start of the study and at all anticipated visits. Women should avoid pregnancy throughout treatment and for up to 4 weeks after the end of medication.

Another risk related to the study concerns the confidentiality of the data. The study team will ensure that the anonymity of the participants is maintained. Participants will be identified only by a participant identification number in all study documents and in any electronic database, with the exception of the CRF, where the participant's initials can be added. The name and any other details
will NOT be included in any electronic study data files. All documents will be stored securely and accessible only by the study team, the Human Research Ethics Committee, and any external auditors of the study. All documents will be stored securely and accessible only by study staff and authorized personnel. The study will comply with the Data Protection Act 2018, which requires that personal data not be kept as identifiable data for longer than is necessary for the purposes in question.

14.7. Benefits

There may be early resolution of symptoms in those who are randomized to certain active treatment arms, but benefit is not guaranteed. As they are drugs already in use for other indications, no harm associated with their use is expected. Although an individual patient may not benefit personally, this study should help future dengue patients by identifying treatments that have the best antiviral effect early in the disease. Participants will be reimbursed for costs associated with travel to the study site and food.

14.8. Communicating

The PI must submit a Semi-Annual Progress Report to the local CEP on the anniversary of the study approval date. In addition, the PI must submit a final study report to the local REC.

14.9. Finances

13.9.1 Funding

The project will be funded with the researcher's own resources received by the INCT project in Dengue and host microorganism interactions.

14.10. Ownership of data

The data generated in this study belongs to the study group as a whole. The final database will be shared between the site PI and key members of the research team.

The database may be shared with researchers not directly involved in this study after the publication of the main article. The criteria for authorship will be consistent with international guidelines (http://www.icmje.org/#autor).

14.11. Publication Policy

The investigators will be involved in reviewing the draft manuscripts, abstracts, press releases, and any other publications arising from the study. Authorship will be determined according to the guidelines of the International Committee of Medical Journal Editors (ICMJE) and other contributors will be acknowledged. The results of the study will be summarized in lay language, both in English and in the language(s) commonly spoken at the study sites, and disseminated to key stakeholders, user communities, and patients.

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Appendages

16. 15. Annex 1: Schedule of activities

Procedures	0			1	2	3	4	7	10	14	28
	Pre 1st dose	H0	H1								
NS1 or qPCR (Tracking)	+										
Clinical Assessment	+										
Assent	+										
Inclusion in the study		+									
Clinical and demographic history		+									
Vital parameters (temp, HR, FR, PA). Weight and height (day 1)		+		+	+	+	+	+			
Symptom assessment		+		+	+	+	+	+	+	+	
Adverse events and medications		+		+	+	+	+	+	+	+	
Laboratory routine*		+		+	+	+	+	+	+	+	
Cytokines		+		+	+	+	+	+	+	+	
Buffy coat (leukocytes)		+			+		+	+		+	
Viral load (RT-PCR)		+		+	+	+	+	+	+	+	
NS1 ELISA		+		+	+	+	+	+	+	+	
PSI Administration		+		+	+	+	+				
Phone contact											+

*At inclusion and on days 3, 7, 10, 14, liver function, albumin, urea, creatinine. ß-HCG test will be performed on days 1, and 14 if necessary. Full Blood Count with platelet count, tests will be performed daily or until the primary efficacy endpoint is reached.