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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Index

1. SYNOPSIS *4*

2. LIST OF ABBREVIATIONS *6*

3. BACKGROUND AND RATIONALE *7*

3.1. Proposal **8**

3.2.Pharmacodynamic evaluation **9**

4. OBJECTIVES AND OUTCOMES *9*

5. STUDY DESIGN *10*

5.1. Intervention **11**

6. PATIENT IDENTIFICATION AND RECRUITMENT *11*

6.1. Study participants **11**

6.2. Inclusion criteria **11**

6.3. Exclusion criteria **11**

7. INCLUSION AND PROCEDURES IN THE STUDY *12*

7.1. Virological sampling **13**

7.2. Recruitment **13**

7.3. Screening and eligibility assessment **13**

7.4. Informed consent **13**

7.5. Baseline Assessments (See Appendix 1 for detailed schedule of activities) **13**

7.6. Study days **14**

7.7. Intensive Pharmacokinetic Sampling: **15**

7.8. Management of patients who become ill **15**

7.9. Sample Handling and Retention **15**

8. DISCONTINUATION/WITHDRAWAL OF PATIENTS FROM THE STUDY *16*

8.1. Definition of End of Study **16**

9. SAFETY REPORT *16*

9.1. Definitions **16**

9.2.Causality **17**

9.3. Procedures for recording adverse events **18**

9.4. Classification of laboratory abnormalities **18**

9.5. Procedures for reporting serious adverse events **18**

9.6. Data Security Monitoring Board (DSMB) **19**

10. STATISTICS AND ANALYSIS *19*

10.1. Overview of the adaptive study design and general approach **19**

10.2. Bayesian hierarchical model of viral clearance **20**

10.3. Time variation effects **20**

10.4. Sample size estimation, randomization, and statistical considerations **21**

10.6. Final analysis of the primary result **21**
11. DATA MANAGEMENT ***21***
11.1. Access to data **21**
11.2. Data Processing and Record Keeping **22**
12. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES ***22***
13. ETHICAL AND REGULATORY CONSIDERATIONS ***22***
13.1. Declaration of Helsinki **22**
13.2. Guidelines for Good Clinical Practice **22**
13.3. Approvals **23**
13.4. Patient confidentiality **23**
13.5. Expenses **23**
13.6. Risk **23**
13.7. Benefits **23**
13.8. Communicating **23**
13.9. Finance **23**
13.10. Data ownership **23**
13.11. publishing policy **23**
14. REFERENCES ***24***
15. Annex 1: Schedule of activities ***26***

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).
Bottom	Dengue complication 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 administering pro-resolving molecules is beneficial in experimental dengue. In these experiments, administration of certain anti-inflammatory or pro-resolving 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 SARS-CoV-2) and decreased disease severity in patients with COVID-19, even in the presence of glucocorticoid therapy. Preclinical experiments have also shown 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 in patients with COVID-19. Further studies are needed to evaluate the safety and efficacy of RESOMELAGON in patients with dengue fever.
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; 2. The participant is able to understand the study procedures and provide informed consent; 3. Presents more than 36 and less than 84 hours after the onset of symptoms; 4. Symptoms compatible with dengue infection with a positive antigen test or positive polymerase chain reaction test. Dengue is suspected by a report of fever and at least 2 other signs or symptoms of the disease, including myalgia, arthralgia, headache or conjunctivitis. **Exclusion Criteria** : 1. Have 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* upon 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 the rib cage) ALT: Alanine aminotransferase; ULN: Upper limit of normal; GFR: Glomerular filtration rate, estimated by the 2021 *Chronic Kidney disease epidemiology collaboration (CKD-EPI) formula* with the serum creatinine value, gender and age of the participant.	
Planned sample size	60 Participants in each arm of the study. 120 participants in total	
Planned Study Period	2 years for total trial duration and 28 days for individual patient involvement	
Interventions	**RESOMELAGON 100mg PO once daily** + standard treatment	
Control	Placebo + standard treatment	
Justification	a. To evaluate the safety of RESOMELAGON in patients infected with dengue fever. The drug has been studied in healthy individuals and patients with rheumatoid arthritis and COVID-19 with favorable safety parameters, but never in dengue fever. Therefore, safety data are needed for future phase 3 trials. b. To assess trends in the efficacy 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 that is prevalent both in Brazil and in other developing countries.	
	Objectives	**Outcome**
Primary security	To evaluate the safety of RESOMELAGON in patients infected with dengue fever	Frequency of adverse events and comparison of hematological, biochemical and virological parameters.
Primary efficacy	Efficacy of RESOMELAGON in reducing disease duration	Time to disease resolution as defined by a composite outcome: a. Patient afebrile for 48 hours with or without antipyretic medication; 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 study patients
	Efficacy of RESOMELAGON in reducing plasma extravasation	Evidence of pleural effusion OR evidence of ascites OR pericardial effusion OR incidence of hemoconcentration (increased hematocrit of >10% from baseline or elevated for age and sex)
	Efficacy of RESOMELAGON in reducing dengue hospitalization or prolonged observation in the emergency department, both of which represent a burden on healthcare systems	Incidence of stays of more than 12 hours in the emergency department or hospital admission
Tertiary	To evaluate mechanisms that may explain the mechanism of action of RESOMELAGON in patients infected with dengue fever	Measurement of cytokines and chemokines and other parameters involved in inflammation and resolution, leukocyte testing for microvesicles and other laboratory parameters considered potentially significant.

2. LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ADLs	Daily activities
CEP	Research Ethics Committee
CRF	Case Report Form – clinical research form
CTCAE	Common terminology criteria for adverse events
DSMB	Data Safety and Monitoring Board
EA	Adverse Event
EAG	Serious Adverse Event
EDTA	Ethylenediaminetetraacetic acid
PPE	Personal protective equipment
EV	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
IP	Principal Investigator
PK	Pharmacokinetics
POP	Standard Operating Procedure
PSI	Product Under Investigation
FROG	Adverse reaction
RAG	Serious Adverse Reaction
RT-qPCR or qPCR	Real-time quantitative polymerase chain reaction
SAR-CoV2	Severe acute respiratory syndrome coronavirus 2
TCLE	Free and Informed Consent Form
GFR	Estimated Glomerular Filtration Rate
TNF	Tumor necrosis factor
U&E	Urea and Electrolytes

3. BACKGROUND AND RATIONALE

The situation for the treatment of socially determined diseases caused by arboviruses, which in Brazil mainly include Dengue, Chikungunya, Yellow Fever and Zika, is quite bleak . In fact, in 2024, we had the worst epidemic in the country's history, with more than 16 million cases and 5 thousand deaths. Although some preventive measures are beginning to be available for some arboviruses, there are no antivirals with demonstrated clinical effect for these diseases. The experience with COVID-19 has clearly shown the beneficial effect of treatment with antivirals (e.g. Paxlovid) and anti-inflammatories (e.g. Glucocorticoids) on 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.

Over the past two decades, Brazil has been facing constant ***dengue epidemics*** with increasing numbers of cases of illness and death, according to data from the Ministry of Health (https://www.gov.br/saude/pt-br/assuntos/saude-de-aaz/d/dengue). Although there is 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 bacteria) [(9)] , but these are also not widely used in our country or even worldwide. Finally, and of interest for the present study, ***there are no antiviral drugs*** or drugs with another mechanism of action approved for use in patients with Dengue. In fact, there are no medications that prevent progression to more severe conditions or that modify the course of the infection in those who develop the most severe forms of the disease.

The inflammatory response is a complex and articulated 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 consists of an initial phase, characterized by the 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 resolution phase of inflammation was considered a passive phenomenon (i.e., occurring due to the absence or catabolism of pro-inflammatory mediators), it is now fully appreciated that specific mediators, signaling pathways and mechanisms must 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 general homeostasis of the body. Since the resolution phase of inflammation is an active process, chronic inflammation can result not only from an overload of pro-inflammatory mediators and mechanisms, but also from an 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 by inhibiting the inflammatory phase and not by pro-resolution mechanisms (https://doi.org/10.1016/j.it.2019.01.007).

Although most dengue virus infections are mild, approximately 5% of cases evolve with an exacerbated inflammatory response that leads to endothelial damage and, thus, plasma leakage. This complication is known as severe dengue and can lead to hypovolemic shock, which is sometimes fatal (https://pmc.ncbi.nlm.nih.gov/articles/PMC9699586). We hypothesize that mechanisms regulating the resolution of inflammation are altered in viral diseases such as dengue (https://doi.org/10.1111/bph.16323 and https://doi.org/10.1111/bph.15164). Thus, the administration of pro-resolution molecules could be useful to slow down or prevent dengue complications. Indeed, the potential benefits of a pro-resolving treatment with Annexin A1 have already been described in a murine model of dengue, reducing thrombocytopenia, vascular permeability and pro-inflammatory cytokines (https://doi.org/10.7554/eLife.73853). Thus, we established a proof of concept that the administration of pro-resolving molecules, which are also anti-inflammatory, are beneficial in the context of dengue.

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 demonstrated that the melanocortin system has several pro-resolution effects. MCR agonists, mainly adrenocorticotropic hormone (ACTH) and αMSH, as well as synthetic analogues such as the AP214 peptide, are anti-inflammatory and have organ-protective effects in several in

vivo models. Furthermore, MCs have been shown to induce pro-resolution effects, such as phagocytosis and efferocytosis, which gives a new aspect to MC-based therapy (https://doi.org/10.3389/fendo.2019.00683). RESOMELAGON has advantageous pharmacology by presenting a biased activation of intracellular signaling pathways, by phosphorylation of extracellular signal-regulated kinases (ERK ½) and intracellular calcium influx without causing cyclic AMP formation. At the functional level, RESOMELAGON modulated inflammatory responses in experimental animals and exerted pro-resolution effects on immune cells, including promotion of efferocytosis and inhibition of 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 type 2 (M2), with this phenomenon playing a central role in the pro-resolution effects of the compound; 3) reduced cytokine release *in vivo* in both LPS-induced systemic inflammatory stress and peritonitis models and 4) significantly accelerated resolution in a peritonitis model when administered therapeutically up to 12 hours after disease induction. Furthermore, 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 doses. (https://www.jimmunol.org/content/194/7/3381)

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 capable of significantly delaying the onset of clinical signs, such as weight loss, conjunctivitis, hair ruffling and reduced locomotor activity, compared to the control groups (MOCK and vehicle). In addition, the treatment was effective in preventing thrombocytopenia, observed by maintaining the number of platelets at levels significantly higher than those of the untreated groups. Additionally, plasma biochemical analyses 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 a significant impact on plasma viral titers, as demonstrated by the quantification of viable viruses by titration assay in Vero CCL81 cells. 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.

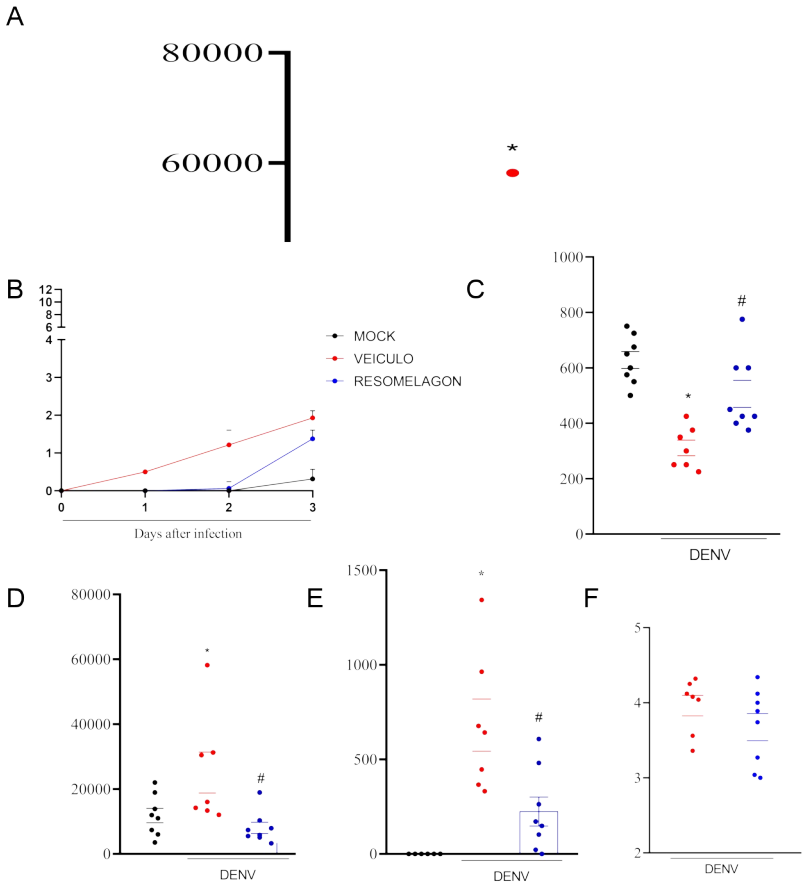

Figure 1: Treatment with RESOMELAGON **delays the onset of clinical signs, prevents thrombocytopenia, and reduces the production of inflammatory mediators without impacting viral titers in plasma. A)** Experimental scheme: A129 mice (deficient for type I interferon receptor) were infected with 2×10^4 PFU of DENV-2 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 erection, and reduced movement. **C)** Platelet count expressed as platelets × 10^3/mL of blood. **DE)** 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 as 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 healthy male volunteer groups (48 men) and one female group of eight healthy postmenopausal women (defined by at least a two-year period of amenorrhea and a follicle-stimulating hormone (FSH) level >30 IU/L) received a single ascending dose with RESOMELAGON/placebo administered as an oral suspension of PSI dissolved in SyrSpend ALKA under fasting conditions. The dose range tested in men was 15 to 800 mg. The dose tested in women was 400 mg.

Pharmacokinetic (PK) results showed that in male participants across 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 C_{max} (maximum plasma concentration) and AUCs (area under the curve) (<36%). The median $t_{1/2}$ 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. 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 regarding the effect of gender.

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 related to study treatment: 21 of these events occurred following administration of 200 to 800 mg of RESOMELAGON and one event occurred following administration of placebo.

The most frequent treatment-related TEAEs were gastrointestinal disorders (19 events reported by fourteen (14) subjects): nausea (7 events), abdominal pain (5 events), vomiting (3 events), diarrhea (2 events), abdominal distension (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 a consistency and increased taste of the study drug. This change in consistency and taste of the suspension may be involved in this effect. Among the TEAEs considered unrelated, headache was reported by seven (7) subjects. Other TEAEs were sporadic.

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

No RESOMELAGON-associated increases in QTcF (the Fridericia-corrected QT interval) or changes in any other cardiac parameters were identified during continuous 24-hour assessment of 1000 HZ ECG Holter 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) was not reached. At the maximum administered dose (MAD), the exposure achieved was more than 10x above the expected exposure level to achieve therapeutic efficacy. Therefore, it was decided not to further 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 given once daily for 14 days. Three trials of 12 participants (9 active; 3 placebo) were dosed with the same formulation used in part 1 of the study. The dose levels tested were 50 mg, 100 mg, or 200 mg with matching placebo.

Pharmacokinetic evaluation showed that **C_{max} was observed between 1 and 2.5 hours post-dose** , regardless of the day of administration. Steady state was reached by day 7. At steady state, C_{max} at the 50 mg dose level was approximately 180 ng/mL (group mean), which is expected to be the exposure level required to induce anti-inflammatory efficacy. Dosing at the 100 mg dose level achieved C_{max} of approximately 400 ng/mL (group mean) 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 mean) 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, the exposure at the 200 mg dose levels, based on Cmax , is up to 5x and, for the highest exposed individual, more than 7x 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 events related to gastrointestinal disorders were observed following administration of the investigational drug (two (2) in the same subject at the 50 mg dose level (one episode of diarrhea and one episode of abdominal cramping, two (2) in the same subject at the 200 mg dose level (one episode of nausea and one episode of vomiting). Among the TEAEs

considered unrelated, nine (9) subjects reported headache, seven (7) treated with 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. Evaluation of QTcF from a continuous 1000 Hz Holter ECG recording performed on day 14 and compared to day 1 (baseline) is still ongoing based on the exposure/response statistical analysis. No clinically significant individual QTcF values were observed at any time point in this study on the repeated standard 12-lead safety ECG.

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

Based on the safety assessment, it can be concluded that the MTD was not reached. At the maximum administered dose (MAD), the exposure was 5x above what would be expected to be the exposure level to achieve therapeutic efficacy. Therefore, it was decided not to further escalate the dose above 200 mg per day.

Consequently, RESOMELAGON has an excellent safety profile, allowing its administration in patients at doses of 50 mg or 100 mg for four weeks. Additional information about RESOMELAGON, including its physicochemical properties and the results of nonclinical in vitro and in vivo pharmacology, pharmacokinetic and toxicology studies, is presented in the Investigator's Brochure.

3.3. Phase 2 clinical trial in COVID-19

A phase 2, double-blind, placebo-controlled 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 for the occurrence of gastrointestinal events. There were also no significant laboratory abnormalities. Although preliminary, a 2-day reduction in the 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 hospital stay from 7 (IQR 5, 14) in the placebo group to 6 (IQR 4, 8) in the RESOMELAGON group was also observed (p=0.038) (https://doi.org/10.1111/bph.17322).

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

In the Phase 2a study, RESOMELAGON was administered as an oral suspension once daily 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 active drug versus placebo in a two-arm design.

- **Part A** : Two groups received active drug (50 mg and 100 mg) versus placebo.
- **Part B** : Participants received active drug/placebo according to one of three study schedules:
    1. 50 mg active drug/placebo
    2. 100 mg active drug/placebo
    3. Either 50 mg or 100 mg of active drug versus placebo.

None of the participants in the active treatment groups had 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 with placebo, primarily due to nausea, constipation, and headache. AEs in the RESOMELAGON-treated groups were predominantly mild, and no serious 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 RESOMELAGON 50 mg and placebo groups. No increases in liver enzymes were observed in participants treated with RESOMELAGON 100 mg. Assessment of the 12-lead ECG performed at each visit did not identify clinically significant abnormal changes. In addition, based on assessment of the 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 was a multicenter, randomized, double-blind, placebo-controlled, two-part study of once-daily RESOMELAGON. The study population consisted of participants with highly active RA (CDAI >22) and inadequate response to DMARD therapy (DMARD-IR), but without elevated CRP at baseline. The first part of the study (Part A) was intended to assess the preliminary safety and efficacy of 4 weeks of 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 of once-daily RESOMELAGON. The study population consisted of newly diagnosed patients with highly active RA (CDAI >22) without elevated CRP at baseline. The primary objective was to evaluate the safety and efficacy of 12 weeks of 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 (TEAEs) were observed between treatment groups (44.4% and 42.2%). Overall, treatment-related TEAEs were reported in 11.1% of the RESOMELAGON group versus 6.3% of the placebo group. Treatment-related TEAEs 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 changes from baseline in mean, median, or maximum/minimum values in safety parameters (chemistry/hematology), vital signs, physical examinations, or ECG. Some changes from normal to abnormal were noted in categorical laboratory parameters, and a few individual values were reported as TEAEs (none considered drug-related).

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

4. OBJECTIVES AND OUTCOMES

	Objectives	Outcomes	Time of outcome assessment
Primary security	To evaluate the safety of RESOMELAGON in patients infected with dengue fever	Frequency of adverse events and comparison of hematological, biochemical and virological parameters.	Days 0- 28
Primary efficacy	Efficacy of RESOMELAGON in reducing disease duration	Time to disease resolution as defined by a composite outcome: a. Patient afebrile for 48 hours with or without antipyretic medication; 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	Days 0-14

		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 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 (increased hematocrit of >10% from baseline or elevated for age and sex)	Days 0-14
	Efficacy of RESOMELAGON in reducing dengue hospitalization or prolonged observation in the emergency department, both of which represent a burden on healthcare systems	Incidence of stays of more than 12 hours in the emergency department or hospital admission	Days 0-14
Tertiary	To evaluate mechanisms that may explain the mechanism of action of RESOMELAGON in patients infected with dengue fever	Measurement of cytokines and chemokines and other parameters involved in inflammation and resolution, leukocyte testing for microvesicles and other laboratory parameters considered potentially significant.	Days 0-14

5. STUDY DESIGN

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

5.1. Intervention

- RESOMELAGON 100mg orally once a day
- Placebo

The investigational product (ISP) will be administered for 5 days. The first dose will be administered at enrollment and then daily in the morning on an empty stomach (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.

6. PATIENT IDENTIFICATION AND RECRUITMENT

6.1. Study participants

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

6.2. Inclusion criteria

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

6.3 Exclusion criteria

1. Have 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* upon 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 the rib cage)
ALT: Alanine aminotransferase; ULN: Upper limit of normal; GFR: Glomerular filtration rate, estimated by the 2021 *Chronic Kidney disease epidemiology collaboration (CKD-EPI) formula* with the serum creatinine value, gender and age of the participant.

7. INCLUSION AND PROCEDURES IN THE STUDY

Patients with a suspected diagnosis of acute arbovirus disease who come from primary care units or emergency care units will be informed of the study and the possibility of undergoing diagnostic testing. Dengue fever is suspected by reporting fever and at least 2 other signs or symptoms of the disease, including myalgia, arthralgia, headache, or conjunctivitis. Patients who can be included will be offered the NS1 rapid test and RT-PCR test for the diagnosis of dengue fever, if these tests have not already been performed. A rapid test for COVID-19 may be performed 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 may occur at any time up to 84 hours after the onset of symptoms, but inclusion and randomization must 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 included in the study after written informed consent.

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

During the initial visit, participants will be asked about their symptoms and medical history. The patient will undergo a physical examination and blood samples for routine hematology and biochemistry and other baseline investigations. After enrollment 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) before you receive your first dose of study medication. The first dose of RESOMELAGON or placebo will be administered under observation in the clinical research unit. For subsequent doses, blood collection and medication administration will be performed during daily visits (at home or at the clinical research unit) under the supervision of study staff.

7.1. Virological sampling

Serial blood samples will be collected according to the sampling schedule by trained study personnel following an established standard operating procedure (SOP). Daily sampling will be performed by study personnel at the participant's home or at the study site. Samples will be kept cool and subsequently stored at -80°C as detailed in the SOP. Transportation times will be recorded, as well as the time for 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 subtype on viral load and response to treatment. This will be done using whole genome sequencing technology or specific PCRs to determine subvariants.

7.2. Recruitment

Potential patients with symptoms suggestive of acute arboviral disease will be invited to participate in the study. Dengue fever is suspected by reporting fever and at least 2 other signs or symptoms of the disease, including myalgia, arthralgia, headache or conjunctivitis. Leaflets will be distributed at primary health care units and/or emergency care centers informing about the study. We will also publicize the study through social media. Participants can contact the research group for more information about the study. All procedures and follow-up of participants will take place at the reference Clinical Research Units where the study is taking place. If the patient has a compatible clinical picture, but less than 36 hours after the onset of symptoms, he or she may be recruited for later inclusion after 36 hours of symptoms.

7.3. Screening and eligibility assessment

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

7.4. Informed consent

Written versions of the participant information and 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 restrictions of the protocol; and the known side effects of the drugs being evaluated 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 their future care and without obligation to provide a 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 provided and obtained informed consent. The person obtaining consent must be appropriately qualified and experienced and must have been authorized to do so by the site PI. One copy of the informed consent signed and dated by the participant and the researcher who obtained consent will be given to the patient and the other copy will be kept on file at the study site.

7.5. Baseline Assessments (See Appendix 1 for detailed schedule of activities **)**

A physical examination, vital signs, oxygen saturation, symptom review, and basic demographic data will be recorded. If there is no medical reason for exclusion, the participant will be enrolled, randomized, and receive the first dose of the drug for which he or she is randomly assigned. A total of 40 mL of venous blood will be collected at this time for specific study-related assessments. The participant may be discontinued before the next dose if laboratory abnormalities result in a contraindication. If a complete blood count and biochemistry were obtained within 36 hours of study enrollment, the results may be used to determine whether there is a contraindication to participation in the study.

Baseline laboratory tests:

- 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 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 obtained 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: The evaluations of Cytokines, 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.

7.6. Study days

7.6.1. Day 1

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 ratios will be uniform for participants (1:1). Only the designated study team (unblinded team) will be allowed to access the envelopes for patient allocation.

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

7.6.2. Days 2 to 7, 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:

- Eligibility check and treatment assessment (administration of medications taken will be observed by study personnel and the time recorded (days 2-5).
- Axillary temperature recorded twice daily by study staff or participant (information collected in a diary and reviewed daily by study staff) only through day 7.
- Study staff complete a brief symptom checklist every day
- Assessment 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 the schedule (appendix 1).
- Blood samples collected to assess cytokine levels , leukocyte testing (buffy-coat), RT-PCR and NS1, according to the schedule (appendix 1).

* See Appendix 1 for detailed schedule of activities.

7.6.3. Day 2 8 (1 month) assessment

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

- Study staff complete a brief symptom checklist ;
- The participant will be asked about EAs and MCs;
- Participation in the study will be completed.

7.7. Management of patients who become ill

During the study, although it is unlikely since we are recruiting patients at low risk of developing more severe disease, the patient may deteriorate clinically or may develop a new intercurrent illness or potentially a side effect related to the study medication. Any patient who develops difficulty with activities of daily living or worsening of their symptoms will be referred to the project team physicians for further evaluation. Clinical deterioration in this study may be due to several factors, including progression of dengue disease, development of complications such as shock, adverse drug reactions, among others. Therefore, a full clinical assessment will

be performed by the study physician. Based on this assessment, they may be referred to an emergency department, re-examined the following day, or asked to update the research team frequently by cell phone about their well-being.

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

Emergency care unit physicians will be responsible for patient care, but the research team will continue to monitor patients' progress in the hospital. Treatment for dengue 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 that may require dose adjustment or discontinuation of therapeutic or clinical intervention. These tests are also important for clinical follow-up of the study participant. The responsibility for evaluating and acting on the results of the blood tests will lie with the study team. They will use their clinical judgment in evaluating the participant, making further tests, and referring the participant to the study. At any time during the study, additional laboratory tests may be performed on the participant if clinically indicated by the research team based on symptoms or previous laboratory abnormalities.

7.8. Sample Handling and Retention

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

Blood samples for virus detection will be processed using validated quantitative real-time polymerase chain reaction (qPCR) to detect dengue, according to the study SOP. The kit to be used is a commercially available kit (Biomol ZDC kit) distributed by IBMP (Instituto de Biologia Molecular do Paraná, FIOCRUZ). Samples will be retained in accordance with local ethical regulations and approvals. Consenting patients may withdraw their consent until the study is completed. To do so, participants must make a written request, sign and date it. 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 may be used for further studies related to 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 tests beyond those indicated in this study will be performed only after obtaining permission from the ethics committee and applying a new ICF if deemed necessary.

8. DISCONTINUATION/WITHDRAWAL OF PATIENTS FROM THE STUDY

Participants may choose to stop treatment and/or study assessments, but may remain under study follow-up. Participants may also withdraw their consent, meaning they wish to withdraw from the study completely. In the case of withdrawal from treatment and active follow-up, the following options for a phased 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 to access their medical records and any relevant hospital data that are recorded as part of routine standard of care; i.e. CT scans, blood results and disease progression data, etc.

c) Patients withdraw, but do not allow the collected data and samples to be used.

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

- Pregnancy;
- Ineligibility (arising during the study or retrospectively having been overlooked in screening);
- Significant deviation from protocol;
- 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 monitored by the study clinical team until the final outcome of the reason for discontinuation, whether due to illness and/or pregnancy.

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

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

8.1. Definition of End of Study

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

9. SAFETY REPORT

9.1. Definitions

Adverse Event (AE)	Any adverse 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 with the study drug qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: ● results in death; ● is a threat to life; ● requires hospital admission or extension of existing hospitalization; ● results in persistent or significant impairment/disability; ● 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 "serious" 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 were more serious. Hospitalization is defined as a formal, unplanned admission, even if the admission is a precautionary measure for continued observation. The patient must be admitted 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 include: emergency room visits, outpatient surgery, pre-planned or elective procedures for a pre-existing condition (provided that the condition did not worsen during the trial treatment or anticipated due to worsening symptoms), and for the purposes of this study, being admitted for influenza isolation.
Serious Adverse	An adverse event that is serious and, in the opinion of the reporting investigator, is reasonably likely to be due to one of the study treatments, based on the information provided.

Reactions (SAD)	

NB: To avoid confusion or misunderstanding about the difference between the terms SAE and “serious” grade 3 AE, the following clarification note is provided: “Serious” is often used to describe the intensity of a specific event, which may be of relatively minor medical significance. SAE is the regulatory definition provided above.

Any pregnancy that occurs during the clinical trial and the outcome of the pregnancy should be recorded and monitored for congenital abnormalities or birth defects, in which case it would fall within the definition of SAE.

9.2. 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 time after administration of the study drug). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
It is unlikely to be related:	There is little evidence to suggest that there is a causal relationship (e.g., the event did not occur within a reasonable time after administration of the study drug) or another reasonable explanation for the event (e.g., the patient's clinical condition, other concomitant treatment).
Unrelated:	There is no evidence of any causal relationship.

9.3. 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 inclusion and during participation in the study 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 or not attributed 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 considered related to the study drug, as judged by a qualified medical investigator, will be followed until resolution or the event is considered stable. All such cases will be appropriately reported and discussed with the company that produces the PSI.

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 because of what he or she perceives as an intolerable AE. If any of these occur, the patient must undergo a final study evaluation and receive appropriate care under medical supervision until symptoms resolve or the condition becomes stable. Abnormalities that are not clinically significant will not be graded or recorded on the CRF.

9.4. Classification of laboratory abnormalities

Abnormal laboratory findings detected during the study or present at baseline and worsening after taking study medication will be reported as AEs or SAEs. If considered abnormal, the values will be graded according to CTCAE v5.0. AEs of severity grade 3 or higher or SAEs will be recorded on the CRF and will be followed until they are grade 2 or lower, return to baseline, or considered permanent. For laboratory results that are not available on 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 assigned to the best of one's ability. Abnormalities that are not clinically significant will not be graded or recorded on the CRF.

9.5. Procedures for reporting serious adverse events

All SAEs detected by the site investigator must 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 resolves, is considered stable/permanent, or results in death. A final status should be determined for any ongoing SAEs at the study end date.

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

9.6. Data Security Monitoring Board (DSMB)

An independent Data Safety Monitoring Board (DSMB) will be established, consisting of qualified volunteers with the necessary knowledge of clinical trials. The DSMB will receive summary reports of the study as defined by the charter 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 concerns, additional information or formal interim analyses may be requested by the DSMB.

The DSMB will formally meet at the following times:

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

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

10. STATISTICS AND ANALYSIS

10.1. 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, mainly in reducing the duration of the disease.

The sample size estimate was calculated by a power analysis *using* data from the literature regarding the mean time until resolution of symptoms and laboratory abnormalities caused by the disease (https://doi.org/10.1371/journal.pntd.0001309): Time until resolution of fever in the placebo group of 136 h and variation of approximately 20% (5.6±1.1 days), time until 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. Considering also an effect size of 1 day in reducing the duration of the disease, power of 80%, alpha of 5% and loss of 10%, we arrive at a sample size of 120 (60 who will receive RESOMELAGON and 60 who will receive the placebo).

Participant randomization will be performed using a centralized system in which only authorized individuals will have access to the application to allocate patients to the placebo or RESOMELAGON group. The webapp will be password protected . All randomization activities will be tracked in the webapp and assigned a timestamp along with the patient's anonymized study code, age and sex.

10.2. Statistical analysis overview

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

For safety analyses and secondary outcomes, the results will be tabulated comparing the treatment with the placebo group. Integer 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. The normality of variables will be tested with the Shapiro-Wilk test, while equality of variance will be analyzed with the F or Bartlett test. Differences in mean or median will be assessed by Wilcoxon, Kruskal-Wallis, 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 associations between continuous or integer variables. Linear and generalized linear models will be used for multivariate structures, and data with temporal or spatial relationships will be explored with random effects models or generalized estimating equations. Estimates of time until the occurrence of an event will be made using Cox models or equivalent non-parametric models. The significance level will be 5%.

11. DATA MANAGEMENT

11.1. 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 regulatory compliance. Outcome data and treatment assignment data will be made available for real-time analysis.

11.2. Data Processing and Record Keeping

Clinical trial data will be recorded on CRFs developed for the trial and entered into a password-protected database by the local trial PI, research nurse, or designee. The trial database will be built on RedCap, a clinical data management system that complies 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 trial database will include internal quality checks to identify data that appears inconsistent, incomplete, or inaccurate.

Measures 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 informed consent forms) 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 data and user activity in the database. All electronic data will be stored on secure servers that are backed up daily, with off-site storage weekly.

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

12. 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 performed 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 in accordance with the Research Site SOPs.

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

13. ETHICAL AND REGULATORY CONSIDERATIONS

This study will be conducted in patients who are unlikely to develop serious illness. The drugs being investigated may benefit the patient; that is, they may shorten the duration of symptoms or reduce their severity, but they are unlikely to have a significant adverse effect on the patient's illness and subsequent health. 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 should any health problems arise as a result of their participation in the study for as long as necessary.

Women who are pregnant, actively trying to become pregnant, or breastfeeding will not be allowed 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. If pregnancy occurs after enrollment in the study, the participant will be discontinued and followed until the pregnancy is complete.

13.1. Declaration of Helsinki

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

13.2. 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).

13.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed publicity material 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.

13.4. Patient confidentiality

The study team will ensure that patient anonymity is maintained. Patients will be identified only by a patient identification number in all study documents and in any electronic databases. 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 is necessary for the purposes in question.

13.5. Expenses

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

13.6. Risk

Resomelagon is well tolerated and safe, and has been tested in normal subjects, patients with rheumatoid arthritis, and patients with moderate COVID-19. The most common side effects include those associated with the gastrointestinal tract, including nausea, vomiting, and diarrhea, and are mostly mild. These are generally mild and resolve without the need for medical intervention. Risks associated with blood withdrawal 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 contraception. Pregnancy tests will be performed on all participants who may become pregnant before the start of the study and at all scheduled

visits. Women should avoid becoming pregnant throughout treatment and for up to 4 weeks after stopping the medication.

Another risk related to the study concerns data confidentiality. The study team will ensure that the anonymity of participants is maintained. Participants will be identified only by a participant identification number in all study documents and in any electronic databases, with the exception of the CRF, where the participant's initials may be added. The name and any other details

Identifying data will NOT be included in any electronic study data files. All documents will be stored securely and only accessible by the study team, the Human Research Ethics Committee and any external study auditors. All documents will be stored securely and only accessible by the study team and authorised personnel. The study will comply with the Data Protection Act 2018, which requires that personal data should not be kept as identifiable data for longer than is necessary for the purposes in question.

13.7. Benefits

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

13.8. Communicating

The PI must submit a Semiannual Progress Report to the local IRB on the anniversary of the study approval date. In addition, the PI must submit a final study report to the local IRB.

13.9. Finance

13.9.1 Financing

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

13.10. Data ownership

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 publication of the main article. Authorship criteria will be consistent with international guidelines (http://www.icmje.org/#author).

13.11. Publication policy

Investigators will be involved in reviewing 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. Study results will be summarized in layman's terms, both in English and in the language(s) commonly spoken at the study sites, and disseminated to key stakeholders, user communities, and patients.

Appendices

15. Annex 1: Schedule of activities

	Triag	1	2	3	4	5	6	7	10	14	28
NS1 or qPCR (Tracking)	+										
Clinical Evaluation	+										
Consent	+										
Inclusion in the study		+									
Clinical and demographic history		+									
Vital parameters (temp, HR, RR, BP). 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		+	+	+	+	+					
Telephone contact											+

*At inclusion and on days 3, 5, 7, 10, 14, liver function tests, albumin, urea, creatinine and ß-HCG will be performed on days 1, 2 and 14 if necessary. For the CBC with platelet count, tests will be performed daily or until the primary efficacy endpoint is reached.