# Trial Title:

**Effectiveness of Nebulized Mild Hypertonic Saline Solution in Children with Mild-to-Moderate RSV Bronchiolitis: randomized, open-label intervention study**

# HYPERBRO

**Internal Reference Number / Short title:**

**Effectiveness of Mild Hypertonic Saline in Mild-to-Moderate Bronchiolitis (HYPERBRO)**

**Date and Version No:** 27 February, 2024, V2.0

## 1. KEY TRIAL CONTACTS

| | |
|---|---|
| **Sponsor** | Gerolymatos International SA<br>Asklipiou 13, 145 68 Kryoneri, Greece<br>Phone: +30 210 35 00 800<br>E-mail: info@gerolymatos-int.com |
| **CRO** | Clinres farmacija d.o.o.<br>Srebrnjak 61, 10000 Zagreb, Hrvatska<br>Phone: +385 1 23 96 900<br>E-mail: info@clinres-farmacija.hr |

## 2. SYNOPSIS

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|---|---|
| Trial Title | **Effectiveness of Nebulized Mild Hypertonic Saline Solution in Children with Mild-to-Moderate RSV Bronchiolitis: randomized, open-label intervention study** |
| Internal ref. no. (or short title) | **Effectiveness of Mild Hypertonic Saline in Mild-to-Moderate Bronchiolitis (HYPERBRO)** |
| Sponsor | Gerolymatos International SA, Asklipiou 13, 145 68 Kryoneri, Greece |
| Clinical Phase | IIIb |
| Trial Design | **Randomized, open-label, active-control intervention study** |
| Trial Participants | Children with mild-to moderate RSV bronchiolitis aged 1-24 months |
| Sample Size | 110 infants and children (1-24 months of age) |
| Planned Trial Period | 20 months trial period, 6 days intervention period, 14 days follow-up |

| Planned Recruitment period | FPFV 01Sep23, LPFV 01May25 | | |
|---|---|---|---|
| | Objectives | Outcome Measures | Timepoint(s) |
| Primary | Compare the percentage of patients that achieved a reduction of 50% in Wang clinical severity score between groups | 50% reduction in Wang clinical severity score | Baseline to Day 3 |
| Secondary | 1) Compare the clinical severity score, temperature, respiratory rate, $SpO_2$ and heart rate between groups | 1) changes in clinical severity score, temperature, respiratory rate, $SpO_2$ and heart rate | 1) Baseline to Day 1 (after 2 h) |
| | 2) Compare the clinical severity score, temperature, respiratory rate, $SpO_2$ and heart rate between the two groups | 2) changes in clinical severity score, temperature, respiratory rate, $SpO_2$ and heart rate | 2) Baseline to Day 7 |
| | 3) Compare the discharge rate or hospital admission rates between groups | 3) Discharge rate and hospital admission rate | 3) after 2 h of observation of day 1 and over the complete 7 days treatment period |
| | 4) Compare the burden of illness in both groups | 4) persistent cough, number of unscheduled visits and number of lost days of work of caregivers | 4) complete 7 days treatment period |
| | 5) Compare frequency of adverse events between group | 5) Adverse events number, severity, association with treatment | 5) Complete 7 days treatment period |
| **Exploratory Objectives** | To compare the improvement in expiratory variability index (EVI) a marker of bronchoconstriction in infants and small children measured using the Ventica® device between groups | Recordal of EVI measurements using the Ventica® device | Day 1, Day 4, Day 6 (overnight measurements extending to Day 2, Day 5 and Day 7, respectively) |
| Intervention(s)<br>• IMD(s) | Sinomarin® Babies single use vials, hypertonic saline (2.3% NaCl), 5 mL, nebulized inhalations | | |

| | |
|---|---|
| • nIMP(s)<br><br>• Other intervention(s) | Bronchodilators (salbutamol or racemic epinephrine), inhaled corticosteroids (budesonide) or parenteral corticosteroids (methylprednisolone), antipyretics (paracetamol or ibuprofen).<br>In case of developing concomitant pneumonia: antibacterial medications (cephalosporine antibiotics, beta-lactam antibiotics, or macrolide antibiotics)<br><br>If necessary: nasal suction, oxygen therapy, rehydration (intravenous saline or glucosaline) |
| Comparator | Physiodose, 0.9 % NaCl, 5 mL vials, Laboratoires Gilbert |

## 3. ABBREVIATIONS

| | |
|---|---|
| AE | Adverse event |
| AR | Adverse reaction |
| CRA | Clinical Research Associate (Monitor) |
| CRF | Case Report Form |
| eCRF | Electronic Case Report Form |
| CRO | Contract Research Organisation |
| CT | Clinical Trials |
| CTA | Clinical Trials Authorisation |
| DM | Data Manager |
| DMC/DMSC | Data Monitoring Committee / Data Monitoring and Safety Committee |
| DSUR | Development Safety Update Report |
| GCP | Good Clinical Practice |
| GP | General Practitioner |
| GTAC | Gene Therapy Advisory Committee |
| IB | Investigators Brochure |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonisation |
| IMD | Investigational Medical Device |
| IRB | Independent Review Board |
| EC | Ethics Committee |
| PI | Principal Investigator |
| PIL | Participant/ Patient Information Leaflet |

| | |
|---|---|
| RSI | Reference Safety Information |
| SAE | Serious Adverse Event |
| SAR | Serious Adverse Reaction |
| SDV | Source Data Verification |
| IFU | Instruction For Use |
| SOP | Standard Operating Procedure |
| SUSAR | Suspected Unexpected Serious Adverse Reactions |
| TMF | Trial Master File |
| TSG | Oxford University Hospitals NHS Foundation Trust / University of Oxford Trials Safety Group |

## 4. BACKGROUND AND RATIONALE

**Main characteristics of the disease**

Bronchiolitis is a common, occasionally severe viral infection of the lower respiratory tract responsible for significantmorbidity and mortality in children under two years of age[1]. According to World Health Organization bulletin, an estimated 150 million new cases of clinical pneumonia (principally pneumonia and bronchiolitis) occur annually [2]; 11–20 million among them requiring hospital admission. Epidemiologic data show that RSV accounts for about 65 % of hospitalizations due to bronchiolitis [3]. Multiple studies [4–7] have documented variation in diagnostic testing, treatment modalities practiced and their outcomes suggesting a lack of consensus for this common disorder. Likewise, despite the frequency of this condition, there is no unanimously accepted evidence driven treatment approach [8, 9]. Besides supplemental oxygen, fluids and supportive care, treatment options include bronchodilators, epinephrine and corticosteroids [9]. Hypertonic saline (3 % NaCl) is a new agent that has been found to be promising in recent studies [10–20]. According to the latest guidelines clinicians may administer nebulized hypertonic saline to infants and children hospitalized for bronchiolitis [21]. Other treatment options as albuterol (or salbutamol), epinephrine, systemic corticosteroids, chest physiotherapy or antibacterial medications (unless there is a concomitant bacterial infection, or a strong suspicion of one) for infants with a diagnosis of bronchiolitis are not recommended [21]. However, there is no data in the literature about the effectiveness of a 2.3 % NaCl hypertonic saline in RSV bronchiolitis in the population of children where it is recommended according to current guidelines.

**Description of the population to be studied**

Children aged 1-24 months with mild-to-moderate bronchiolitis (with first episode of wheezing associated with a history of upper respiratory tract infection) as evaluated with a Wang score from 3 to 8, visiting the Emergency Hospital Department. Participants have to fulfil inclusion and exclusion criteria defined in Chapter 7.

**Characteristics of the investigational medicinal product(s)**

Sinomarin® Babies is a 100 % natural hypertonic sea water solution (2.3 % NaCl). The product is available in 5 mL single use vials. It is used to relieve nasal congestion, to help thin nasal mucus making it easier to eliminate and to cleanse the nasal cavities helping protect from nasal and further upper respiratory infections and complications. Sinomarin® Babies can be used in babies, both for regular nasal hygiene and congestion relief. Sinomarin® Babies can also be used in a nebulized form to help enhance respiratory function, in cases of acute bronchiolitis.

IMD has a marketing authorisation in the EU, bearing a CE-mark under the European Medical Device Directive (MDD) 93/42/EEC, as amended by the directive 2007/47/EC.

**Summary of risks and benefits**

**Potential risks:** For nasal and respiratory (with nebuliser) use only; each vial is intended for singe use only. Should not be used in case of hypersensitivity to seawater.

**Benefits:** It helps reduce airway edema and thins airway mucus, thus decreasing airway obstruction. In cases of bronchiolitis, it helps enhance respiratory function and prevent lower respiratory tract infections. It is 100 % natural, sterile, free from drug substances, additives and preservatives. It does not induce drowsiness, habit formation, or rebound effect.

**Rationale for the trial**

In the paucity of rigorously controlled studies for such a common disease of infancy as RSV bronchiolitis with the only conditionally recommended treatment by the Guidelines as: “Clinicians may administer nebulized hypertonic saline (HS) to infants and children hospitalized for bronchiolitis (Evidence Quality: B; Recommendation Strength: Weak Recommendation [based on randomized controlled trials with inconsistent findings]” and a lack of a consensus regarding management of bronchiolitis it is decided to provide a trial with a hypertonic saline of 2.3 % NaCl to assess its therapeutic efficacy. Therapeutic efficacy of a nebulized 2.3 % NaCl hypertonic saline for bronchiolitis in children was not yet tested. Its therapeutic efficacy was tested in other clinical respiratory disorders where it showed comparable efficacy to 3 % HS and safety comparable to placebo or 0.9 % saline. It is chosen to provide nebulizations of 5 mL 2.3 % HS both at hospital and at home for infants and children with mild-to-moderate bronchiolitis in need for hospitalization through daily hospital > 72h. To prove superiority, as a comparator it is decided to use nebulizations of 0.9 % saline. The goal is to study primarily the improvement in clinical scores but also look at parameters like readiness for discharge, need for hospital revisit rates and hospitalization rates which would reflect the morbidity, but also persistent cough at Day 7, total number of lost days of work of caregivers for the period, adverse events and as the exploratory variable record lung function (level of bronchial obstruction) using the newest technology available for infants and small children (Ventica® recordings on Day 1 (baseline), Day 4, Day 6).

**Literature references**

See Chapter 21. LITERATURE.

## 5. OBJECTIVES AND OUTCOME MEASURES

| Objectives | Outcome Measures | Timepoint(s) of evaluation of this outcome measure (if applicable) |
|---|---|---|
| **Primary Objective**<br>Compare the percentage of patients that achieved a reduction of 50 % in Wang clinical severity score between tested groups | 50 % reduction in Wang clinical severity score<br><br>Wang clinical severity score would be measured each day according to Wang EE et al. [22] and compared to baseline | Baseline to Day 3 |
| **Secondary Objectives** | | |
| 1) Compare clinical severity score, temperature, respiratory rate, $SpO_2$ and heart rate between groups | 1) changes in clinical severity score, temperature, respiratory rate, $SpO_2$ and heart rate | 1) Baseline to Day 1 (after 2 h) |
| 2) Compare clinical severity score, temperature, respiratory rate, $SpO_2$ and heart rate between two groups | 2) changes in clinical severity score, temperature, respiratory rate, $SpO_2$ and heart rate | 2) Baseline to Day 7 |
| 3) Compare the discharge rate or hospital admission rates between groups | 3) Discharge rate and hospital admission rate | 3) after 2 h of observation of day 1 and over the complete 7 days treatment period |
| 4) Compare the burden of illness in both groups | 4) persistent cough, number of unscheduled visits and number of lost days of work of caregivers | 4) complete 7 days treatment period |
| 5) Compare frequency of adverse events between group | 5) Adverse events number, severity, association with treatment | 5) Complete 7 days treatment period |
| **Exploratory Objectives**<br>To compare the improvement in expiratory variability index (EVI) a marker of bronchoconstriction in infants and small children measured using the Ventica® device between groups | Recordal of EVI, measurements using Ventica® device [23] | Day 1, Day 4 and Day 6 (overnight measurements extending to Day 2, Day 5 and Day 7, respectively) |

## 6. TRIAL DESIGN

This is a randomized, open labelled, active-controlled, parallel design, superiority trial of nebulized hypertonic saline (2.3 % NaCl) added to a standard treatment compared with nebulized saline (0.9 % NaCl) with standard treatment in children with mild-to-moderate RSV bronchiolitis.

This is a single country (Croatia), multicentre trial where children with RSV bronchiolitis will be recruited, randomized and provided with intervention and continuing care if necessary.

Based on the level of severity of bronchiolitis (mild-to-moderate, Wang score 3-8) it is expected from our clinical practice that infants and children will be involved in treatment for 6 days, with regular visits every day for 7 days. Screening visit will be done on the Day 1, together with initial treatment and post-treatment follow-up. Children will come every day during next 5 days (Day 2 to Day 6) for a hospital evaluation and treatment and will have 2 treatment sessions at home each day. At Day 7 only post-treatment follow-up visit will be done or treatment will be provided if necessary.

On Day 1 Patient Information, Informed Consent Form, eligibility and enrolment, together with baseline physical exam, vital signs (temperature, respiratory rate, heart rate), Wang score, $SpO_2$ and 2 hospital nebulization sessions at 0 and 30 minutes (followed by 2 home sessions by the parent upon return to home) will be done. Vital signs, Wang score and $SpO_2$ will be examined at 1 and 2 h after starting nebulization, and before discharge. Potential adverse events will be recorded. If patients do not have nebulisers, the site will provide those with nebulisers during the course of the trial (Day 1 to Day 7). Optionally, provision of Ventica® device will be done together with education for parents/legal guardians on how to use the device during sleep over night to record lung function. On consecutive days (Day 2 to Day 6) a daily physical exam, Wang Score determination, $SpO_2$ and vital signs (temperature, respiratory rate, heart rate) will be done together with 1 hospital nebulization session followed with examination of vital signs, $SpO_2$ and Wang score at 1 and 2 h after starting nebulization, and before discharge. Daily discharge rate or hospital admission rate evaluation will be done. Potential adverse events will be recorded. After discharge every day, 2 home sessions of nebulization will be done. Parents or legal guardians will fulfil subject a dosing diary every day, recording details on nebulization, concomitant medication and adverse events. For each hospital visit, parents or legal guardians will return used vials from the previous day, so that compliance could be measured. At Day 7, physical exam will be done, as well as vital signs, Wang score and $SpO_2$ measurement. Persistent cough will be evaluated together with total number of unscheduled visits for the period, total number of lost days of work of caregivers for the period, adverse events would be recorded. The subject dosing diary will be returned. Ventica® recordings from Day 1 (baseline), Day 4, and Day 6 would be downloaded from the device that parents/legal guardians would return (Day 7). On Day 7 treatment will be provided as needed.

The flowchart of the project is presented in Appendix 1.

## 7. PARTICIPANT IDENTIFICATION

### 7.1. Trial Participants

Infants and children aged 1 - 24 months with a first episode of wheezing and/or mild-to-moderate bronchiolitis will be enrolled in the study.

### 7.2. Inclusion Criteria

Previously healthy children visiting the Emergency Room (ER) and Day Care-Department (DCD) with the following inclusion criteria will be recruited:

1. Age between 4 weeks and 2 years
2. First Episode of Wheezing
3. History of upper viral respiratory tract infection (symptoms of coryza, cough or fever)
4. Children with mild-to-moderate disease as evaluated with the Wang score [22] between 3 and 8.

### 7.3. Exclusion Criteria

The participant may not enter the trial if ANY of the following apply:

1. Any underlying disease, comorbidity or congenital malformation (e.g., cystic fibrosis, bronchopulmonary dysplasia, cardiac or renal disease, etc.)
2. Prior history of wheezing or asthma
3. Children with severe disease as evaluated with the Wang score (> 8)
4. Children with progressive respiratory distress requiring mechanical ventilation
5. Previous treatment with bronchodilators (within last 4 h) or steroids (within 48 h) or nebulized saline solutions (within 12 hours)

## 8. TRIAL PROCEDURES

### 8.1. Recruitment

Previously healthy children during the visit to the Emergency Room (ER) and/or Day Care-Department (DCD) with the clinical presentation of mild-to-moderate bronchiolitis will be selected based on inclusion and exclusion criteria.

Recruitment will occur during bronchiolitis seasons in between September 2023 until May 2025 (during a 20 month period). The investigator will assess infants and children for eligibility and assign a clinical severity score (by Wang et al.) All data will be collected using standardized forms to document pertinent information and physical exam. Child's weight and height, vital signs (body temperature, respiratory rate, heart rate), $SpO_2$ (determined using a pulse oximeter), and severity Score will be recorded. Children will be stabilized with antipyretics (paracetamol or ibuprofen) if necessary (temperature > 38.3$^{o}$C) and/or a nasal suction will be done if the nose is blocked. Patients determined to be in life threatening condition will be immediately managed and referred to intensive treatment, and will not be further considered for study. Other selected respondents who meet the inclusion criteria, after Informed Consent Form has been signed by their parents/legal guardians, will be randomized into two groups.

### 8.2. Screening and Eligibility Assessment

Infants and small children aged 1 - 24 months with symptoms and signs of acute respiratory infection during the RSV season visiting the Emergency Hospital Department will be screened for eligibility. Eligibility assessment will be done based on inclusion and exclusion criteria and obtained Informed Consent Form after providing all relevant information about the trial from parents/legal guardians.

### 8.3. Informed Consent Form

A written Informed Consent Form will be obtained from the parents/legal guardians of the patients prior to the enrolment. The study should be approved by the Central Ethics Committee, Ministry of Health and all sites' Local Ethics Committees, if applicable. The parents/legal guardians must personally sign and date the latest approved version of the Informed Consent Form before any trial specific procedures are performed.

Written and verbal versions of the patient information and Informed Consent Form will be presented to the parents (to at least one of the parents) or legal guardian, detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The parents/legal guardians will be allowed as much time as they wish to consider the information, and the opportunity to question the Investigator, their paediatrician or other independent parties to decide whether they will participate in the trial. Written Informed Consent Form will then be obtained by means of parents/legal guardians, dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Principal Investigator. A copy of the signed Informed Consent Form will be given to the parents or legal guardians. The original signed form will be retained at the trial site.

### 8.4. Randomization

Block randomization method will be used to stratify patients into blocks of 3 each, each comprising of 3 patients. The 2:1 sequence of administration will be assigned to the subject by randomization module implemented to the study eCRF. Blocked randomization sequence list will be produced by the DM and documented in data management plan.

Randomization button and sequence will be validated by using database validation forms.

Additional training on randomizing subjects will be provided by DM prior to the enrollment. Steps will be explained in the eCRF user manual.

### 8.5. Blinding and code-breaking

As this is an open-label trial, no blinding or code-breaking would be implemented in this trial.

## 8.6. Baseline Assessments

**DAY 1**

Baseline assessment will be done after parents/legal guardians sign the Informed Consent Form on the first day of trial in Day Hospital Department (DHD). The assessment will comprise:

- All patient Information
- Baseline physical exams, Wang Score determination, $SpO_2$ and vital signs (temperature, respiratory rate, heart rate), weight, height
- Randomization
- Treatment, which will consist of 2 hospital nebulization sessions at 0 and 30 minutes and two home nebulizations, after discharge. Each nebulization comprises a first nebulization of standard medication (bronchodilator) followed by IMD/comparator nebulization
- The investigator will assess the children's general condition and record the severity score, $SpO_2$ and vital signs (temperature, RR and HR) at 60 and 120 min after the starting nebulisation as well as before discharge for this day
- Discharge or hospital admission evaluation
- Recording of potential adverse events
- Provision of subject diary with instructions
- Provision of nebulizers, if needed
- Provision of Ventica device accompanied with education on how to use the device in patients who will use it

## 8.7. Subsequent Visits and follow-up

Each recruited and randomized subject from both groups will be monitored for 7 days, and during days 2 to 6, they will receive at least one inhalation in the hospital and 2 inhalations at home.

**DAYS 2 – 6**

The treatment will consist of 1 hospital nebulization session and 2 home nebulization sessions. Each nebulization comprises a first nebulization of standard medication (bronchodilator) followed by IMD/comparator nebulization. For each home nebulization, parents or legal guardians will fill out a subject dosing diary. During the clinical visit, the investigator will assess the children's general condition and record the severity score, $SpO_2$ and vital signs (temperature, RR and HR) prior to each hospital nebulization, and at 60 and 120 min after the starting of nebulization, as well as before going home or receiving inpatient treatment.

For each clinical visit, list of assessments that will include the following will be evaluated:

- ✓ eligibility check (they should be excluded if clinical deterioration mandated escalation of therapy and/or support or if needed any unscheduled hospital visit and hospitalization within the next 24 h between visits)
- ✓ assessment of outcome measures (severity score, HR, $SpO_2$, RR)
- ✓ assessments of safety including general (e.g. physical examination), specific safety assessments e.g. adverse event such as heart rate > 200, tremor and worsening of clinical status

- ✓ dispensing of trial product if the two courses of nebulization were not delivered, the product delivery was delayed by 10 min or more (protocol deviations)
- ✓ assessment of compliance with trial intervention (home inhalations) by controlling the number of returned used vials recording of concomitant medications (e.g. bronchodilators, corticosteroids, antipyretics)
- ✓ Discharge or hospital admission evaluation.

**DAY 7**

Treatment will be provided only if needed.

For clinical visit on day 7, the following will be evaluated:

- ✓ outcome measures (severity score, $SpO_2$ and vital signs)
- ✓ discharge or hospital admission evaluation
- ✓ occurrence of adverse events
- ✓ concomitant medication and treatment
- ✓ compliance
- ✓ persistent cough
- ✓ total number of unscheduled visits for that period
- ✓ total number of lost days of work for parents/legal guardians for that period
- ✓ for patients to whom Ventica® device was provided on Day 1, results will be downloaded for the following nights: Day 1 → Day 2, Day 4 → Day 5, Day 6 → Day 7.

**DAY 14**

A follow up will be done via telephone call to assess patient symptoms related to bronchiolitis.

The schedule of assessments of the project is presented in Appendix 2.

### 8.8. Sample Handling

No samples will be taken or analysed for trial purposes during the course of trial. Blood samples will be taken only in the course of the regular clinical management of children.

### 8.9. Early Discontinuation/Withdrawal of Participants

During the course of the trial parents/legal guardians may choose to withdraw early from the trial treatment at any time. This may happen for a number of reasons, including but not limited to:

- The occurrence of what the participant perceives as an intolerable AE.
- Inability to comply with trial procedures.
- Participant decision.

Parents/legal guardians may choose to stop treatment and/or study assessments but may remain on study follow-up.

Parents/legal guardians may also withdraw their consent, meaning that they wish to withdraw from the study completely. In the case of withdrawal from both treatment and active follow up the following options for a tiered withdrawal from the study will be offered;

1) Participants may withdraw from active follow-up and further communication but allow the trial team to continue to access their medical records and any relevant hospital data that is recorded as part of routine standard of care.
2) Participants can withdraw completely from the study and withdraw all data collected up until the point of withdrawal.

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

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures
- Disease progression which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures

The type of withdrawal and reason for withdrawal will be recorded in the eCRF.

If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

### 8.10. Definition of End of Trial

The end of trial is the point at which all the data have been entered and queries resolved.

## 9. TRIAL INTERVENTIONS

### 9.1. Investigational Medical Device(s) (IMD) Description

IMD: Sinomarin® Babies single use vials, hypertonic saline (2.3 % NaCl), 5 mL, nebulized inhalations.

Comparator: Physiodose, 0.9 % NaCl, 5 mL vials, Laboratoires Gilbert, nebulized inhalations.

Both products have marketing authorisation in EU.

**Groups**:

**Group 1**: Infants receiving standard nebulized medication and nebulized 0.9 % NaCl

**Group 2**: Infants receiving standard nebulized medication and nebulized 2.3 % NaCl (Sinomarin Babies)

**Administration of treatments:**

**Day 1:** 2 hospital nebulization sessions and 2 home nebulization sessions

**Days 2-6**: 1 hospital nebulization session and 2 home nebulization sessions

**Day 7**: treatment as needed

Each nebulization comprises a first nebulization of standard medications (bronchodilator) followed by saline nebulization.

#### 9.1.1. Blinding of IMDs

There is no blinding procedure of IMD and comparator in the trial. This is an open label study.

#### 9.1.2. Storage of IMD

Investigational product and comparator will be stored in a secure/locked environment with access provided only to key study personnel who have the appropriate authorization. The investigational product and comparator will be stored separately, according to the manufacturer's recommendations (as defined in the IFU). Both products will be stored at the required temperature and temperature log will be maintained.

#### 9.1.3. Compliance with Trial Treatment

The investigator or his/her designated and qualified representatives will administer/dispense study product only to subjects enrolled in the study in accordance with the protocol. The study product must not be used for reasons other than that described in the protocol. Subject dosing will be recorded on a subject dosing diary. Subjects will be instructed to return all product containers (even if empty) to the study site personnel at each clinic visit. The study site personnel will document compliance in the study source documents.

#### 9.1.4. Accountability of the Trial Treatment

Upon receiving investigational products from the study sponsor, the Investigator or designee will ensure that information on packaging slip matches exactly with what the site has received. The investigator or designee should also check the number of investigational products, lot numbers, etc. It is good practice to update the device accountability log with relevant information immediately.

#### 9.1.5. Post-trial Treatment

There is no provision of the IMD beyond the trial.

### 9.2. Other Treatments (non-IMPs)

There are no non-IMPs in the trial design.

### 9.3. Other Interventions

All subjects will receive standard therapy for bronchiolitis. Standard therapy is bronchodilators (salbutamol or racemic epinephrine), inhalation corticosteroids (budesonide) or parenteral corticosteroids (methylprednisolone).

If the temperature is higher than 38.3$^{o}$C, subjects will receive antipyretics (paracetamol or ibuprofen).

In case of developing concomitant pneumonia, subjects will receive antimicrobial therapy (cephalosporine antibiotics, beta-lactam antibiotics or macrolide antibiotics. If needed, subjects will receive standard oxygen therapy or rehydration (intravenous saline or glucosaline).

## 10. SAFETY REPORTING

The safety reporting window for the trials starts from the time of consent to 30 days after last administration of the IMD.

All AEs occurring at any time during the study will be followed by the Investigator until stable outcome.

### 10.1. Adverse Event Definitions

| | |
|---|---|
| Adverse Event (AE) | Any untoward medical occurrence in a participant to whom a medical device has been administered, including occurrences which are not necessarily caused by or related to that product. |
| Adverse Reaction (AR) | An untoward and unintended response in a participant to an investigational medical device which is related to any dose administered to that participant.<br>The phrase "response to an investigational medical device" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.<br>All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions. |
| Serious Adverse Event (SAE) | A serious adverse event is any untoward medical occurrence that:<br>• results in death<br>• is life-threatening |

| | |
|---|---|
| | • requires inpatient hospitalisation or prolongation of existing hospitalisation<br>• results in persistent or significant disability/incapacity<br><br>Other 'important medical events' may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.<br><br>NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. |
| Serious Adverse Reaction (SAR) | An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided. |
| Suspected Unexpected Serious Adverse Reaction (SUSAR) | A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information for the medicinal product in question set out:<br><br>• in the case of a product with a marketing authorisation, in the approved Clinical Evaluation Report (CER) and Instruction For Use (IFU) for that product |

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

### 10.2. Assessment results outside of normal parameters as AEs and SAEs

Abnormal clinical findings from safety blood tests will be assessed by a medically qualified study member. Laboratory AEs will be assessed using specific toxicity grading scales adapted from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (Corrected Version 2.1 - July 2017).

### 10.3. Assessment of Causality

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

Reasonable Possibility – After consideration of factors including timing of the event, biologic plausibility, clinical judgment, and potential alternative causes, there is sufficient evidence (information) to suggest a causal relationship.

No Reasonable Possibility – After consideration of factors including timing of the event, biologic plausibility, clinical judgment, and potential alternative causes, there is insufficient evidence (information) to suggest a causal relationship. For causality assessments, events assessed as having a reasonable possibility of being related to the study product will be considered "associated." Events assessed as having no reasonable possibility of being related to study product will be considered "not associated." In addition, when the investigator has not reported a causality or deemed it not assessable, Sponsor will consider the event associated. If an investigator's opinion of no reasonable possibility of being related to study product is given, another cause of event must be provided by the investigator for the SAE.

## 10.4. Procedures for Reporting Adverse Events

AEs occurring during the safety window for the trial as defined above that are observed by the Investigator or reported by the participant, will be reported on the trial eCRF, whether or not attributed to trial medication.

The following information will be reported on the eCRF: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

Non-serious AEs considered related to the trial medication as judged by a medically qualified investigator or the Sponsor will be followed up either until resolution, or the event is considered stable.

All SAEs, other than those defined in this protocol as not requiring reporting, must be reported on the SAE Reporting Form to the Sponsor or delegate immediately or within 24 hours of Site Study Team becoming aware of the event being defined as serious.

### 10.4.1. Events exempt from immediate reporting as SAEs

Hospitalisation for a pre-existing condition, including elective procedures planned prior to study entry, which has not worsened, does not constitute a serious adverse event. Also, hospitalisation for procedures and treatments specified within the protocol, and standard supportive care for the disease under study are not SAEs, and do not require SAE reporting.

### 10.4.2. Procedure for immediate reporting of Serious Adverse Events

The Site study team will complete an SAE report form for all reportable SAEs. Where the SAE requires immediate reporting, the SAE report form will be scanned and emailed to sponsor or sponsor delegate immediately i.e., within 24 hours of site study team becoming aware of the event. Conventional SAE form will be completed and send by e-mail. Follow-up SAE information Collection and notification to the Sponsor will occur in the same manner and timelines as for initial SAEs.

### 10.5. Expectedness

Expectedness will be determined according to the approved RSI in the Clinical Evaluation Report (CER).

### 10.6. SUSAR Reporting

All SUSARs will be reported by the Sponsor or Sponsor delegate to the relevant Competent Authority and to the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or Sponsor delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

Principal Investigators will be informed of all SUSARs for the relevant IMD for all studies with the same Sponsor, whether or not the event occurred in the current trial.

### 10.7. Development Safety Update Reports

The Reference Safety Information (RSI) in effect at the start of a Development Safety Update Report reporting period serves as the RSI during the reporting period. For follow-up reports, the RSI in place at the time of occurrence of the 'suspected' Serious Adverse Reaction will be used to assess expectedness.

Sponsor or sponsor delegate will submit DSURs once a year throughout the clinical trial, or on request to the Competent Authority.

## 11. STATISTICS

### 11.1. Statistical Analysis Plan (SAP)

The plan for the statistical analysis of the trial is outlined below. There will be a separate SAP document in use for the trial.

### 11.2. Description of Statistical Methods

Ordinal and nominal variables would be presented as number and proportions, and parametric variables as mean with standard deviation (SD) or as median and interquartile range (IQR) depending on the type of distribution. The baseline values of variables will be compared between groups using chi-square or Fisher exact tests for ordinal and nominal variables and using Student's t-test or Mann-Whitney U test depending on the distribution for parametric variables. The primary analysis for the primary outcome would be done using Kaplan-Meier curve and log-rank test to compare the outcome between groups. Secondary analysis for the primary outcome would be done using Cox proportional hazards regression with the inclusion of demographic variables, baseline severity (clinical score, HR, RR, $SpO_2$, body temperature, EVI score). For the secondary variables and exploratory variable repeated measures analysis of variance would be used for parametric variables and chi-square or Fisher exact tests for ordinal and nominal variables to compare outcomes between groups. $P < 0.05$ would be used in all analyses as the statistically significant. Data analysis will be performed in IBM SPSS Statistics for Windows, Version 24.0.

### 11.3. Sample Size Determination

Estimated number of participants: ~110 patients to be recruited. The sample size was calculated based on the assumption that 70 % of subjects from the active group would reach 50 % reduction of Wang score (primary outcome) compared to 40 % reaching the primary outcome in the control group assuming 2:1 randomization ratio and 15 % dropout rate → 73 active vs. 37 control subjects with the alpha = 0.05 and a statistical power of 80 %.

Sample size and the ratio between active and control group is also based on the potential number of subjects that could be recruited in a single centre study during one RSV season and based on the assumption of the superiority of active treatment also associated with good safety profile. A very conservative dropout rate of 15 % is also calculated.

### 11.4. Analysis Populations

All enrolled and randomized participants would be analysed as intention to treat population (ITT). Also, all participants having all data on all planned visits would be analysed as per protocol population (PP) and compared to ITT population as the sensitivity analysis. All dosed participants (receiving at least one dose of study drug or control) would be analysed for adverse events.

### 11.5. Decision Points

There will be no interim analysis and no decision points are therefore defined.

### 11.6. Stopping Rules

There will be no rule for stopping the trial based on the lack of efficacy or lack of power because there are no other effective treatments available.

### 11.7. The Level of Statistical Significance

The level of significance that will be used for all tests will be $p<0.05$ with the appropriate correction for multiple comparisons.

### 11.8. Procedure for Accounting for Missing, Unused, and Spurious Data.

Randomly missing, unused and spurious data will be completed by the data management team by discrepancy queries. Any data that cannot be completed by site will be handled with the principle Last Observation Carried Forward (LOCF) and these data would then be analysed as though there are no missing data. If missing, unused and spurious data would occur a missing (unused, spurious) follow-up visit value is replaced by (imputed as) that subject's previously observed value, i.e. the last observation is carried forward. To avoid systematic bias only randomly missing, unused and spurious data would be handled that way. The cases with an uncomplete data set, completely missing final visit would be handled using two different analysis; intention-to-treat (ITT) would be compared to per protocol (PP) analysis data as a sensitivity and possible bias analyses.

### 11.9. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

No deviations from the original statistical plan should occur. In case of any, detailed reporting of any deviation(s) from the original statistical plan will be described and justified in the final report, as appropriate.

### 11.10. Health Economics Analysis

No health economics analysis is planned and no data related to that subject would be gathered and analysed.

## 12. DATA MANAGEMENT

Web-based Helios EDC system will be used for randomization, data entry, query management, source data verification and transmit study data. EDC system will assign unique number for each subject. Database and eCRF will be prepared by using protocol and concept CRF by data manager. EDC system will be validated and approved by data management prior to data entry.

The investigator or qualified designee is responsible for the accuracy, completeness, legibility, and timelines of subject data. All data collected for the study should be recorded accurately, promptly, and

legibly. By signing this protocol or using personal eCRF user account, the investigator acknowledges that his/her EDC password and electronic signature is the legally binding equivalent of a written signature.

Access to the eCRF will be strictly password protected and limited to personnel directly participating in the study. Study data manager will be responsible for user access management of the study. Data should be entered into the eCRF completely by authorized and trained personnel. The eCRF must be completed as soon as possible after any evaluation. If data are to be changed due to erroneous input or other reason, an electronic audit trail will track the changes. The eCRFs will be accessible to study monitors and other regulatory auditors if required.

The overall procedures to clinical study data management are described in the data management standard operational procedures which will be listed in the study data management plan. Separate data management plan document will provide detailed descriptions of source documentation, eCRFs, instructions for completing forms, data handling procedures, and procedures for data monitoring.

Manual data review and cleaning will be done by data management to check for discrepancies and to ensure consistency of the data. Query management will be done in Helios EDC to track any changes. Any data change request by data management or study monitors should open an electronic query in EDC. Data reports will be available to the authorized personnel to download in Helios EDC. If automatic edit-checks will be implemented, they should be validated in the database validation form by study DM.

### 12.1. Non-CRF data

A separate printed dosing diary form will be available on site to distribute to parents or legal guardians. The dosing diary will be completed every day by parents or legal guardian. Data on nebulization, concomitant medication and adverse events will also be recorded. At the last hospital visit, on Day 7, parents or legal guardians will return the dosing diary from the previous 6 days, so that the data can be recorded on eCRF.

Ventica® recordings from Day 1 (baseline), Day 4, and Day 6 would be downloaded from the device that parents/legal guardians would return on Day 7. There will be a file upload section in the eCRF for the downloaded recordings from the devices. If there are sensitive subject data present in the downloaded media, trained authorized site staff should blind the subject-sensitive data on the downloaded media.

## 13. QUALITY ASSURANCE PROCEDURES

### 13.1. Risk assessment

In Clinical Evaluation Report, risk factors and side effects associated with hypertonic seawater solutions (2,3 % NaCl, 2,6 % NaCl, 3,1 % NaCl) are described.

Among the adverse events that are associated with nasal saline irrigation solutions and were reported in the literature are the following: Epistaxis, nasal irritation, nasal burning, nasal discomfort, nasal dryness, nasal drainage, nasal mucosal ulcer, nasal congestion, cough, headache, tearing, transient and mild ear pain.

Most frequently reported adverse events are nasal burning, irritation, nausea and inducing crying in babies and young children due to poor tolerance.

Most of these adverse events occurred after the first treatment and were resolved after that.

A risk assessment will be reviewed as necessary over the course of the trial.

### 13.2. Monitoring

Regular monitoring will be performed according to the trial specific Monitoring Plan. The study will be monitored in compliance with the protocol, GCP and the applicable regulatory requirements. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Monitoring visits will be carried out at the sites during the study to ensure that any deviations are reported to the Sponsor. Among other responsibilities, monitor will perform the following duties:

1. Initiate the investigation site: 1 Site Initiation Visit, after all approvals from RA/EC and Sponsor green light have been obtained and after all applicable documentation for start of study have been signed and collected.
2. Perform routine on-site monitoring visits: frequency of Monitoring Visits is defined by the number of subjects enrolled in the study and described in the Monitoring Plan. The Monitor will review the Informed Consents Forms and ensure that the Informed Consent Form procedure has been appropriately carried out. Source data will be reviewed against the eCRF, and it will be ensured that all reportable events have been reported within the applicable timeframes.
3. Conduct close-out activities: 1 Close-Out Visit, when the database lock has occurred, and all outstanding issues have been resolved.

All monitoring activities shall be documented, and a report will be provided to the Sponsor. A summary of key findings will be shared with the Principal Investigator in a Follow-up Letter.

Detailed monitoring procedures will be described in a separate Monitoring Manual.

### 13.3. Trial committees

There are no trial committees foreseen for this study.

#### 13.3.1. Safety Monitoring Committee

There is no Safety Monitoring Committee in this trial.

## 14. PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMD administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Investigators shall not deviate from the protocol without prior approval from the Sponsor, except when it is necessary to protect the life of physical wellbeing of the subject.

Deviations are classified as major or minor:

**Major deviation:** Any deviation that may significantly impact the completeness, accuracy or reliability of key study data or that may significantly affect patient rights, safety or well-being.

**Minor deviation:** Deviation which does not affect the scientific soundness of the research plan or patient rights, safety, or well-being.

Subject specific deviations will be reported in the eCRF.

Non-subject specific deviations will be reported to the Sponsor on a protocol deviation form.

All deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

Deviations will be reported to the Ethics Committee and Competent Authority when the deviation:

- Affects the subject's rights.
- Affects the subject's safety and well-being.
- Affects the scientific integrity of the clinical investigation.

Based on the analysis and the assessment of the Sponsor and the CRO, corrective and preventive actions will be created and applied to avoid any future deviation of the same sort.

## 15. SERIOUS BREACHES

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the appropriate Regulatory authority and the relevant functions within seven calendar days.

## 16. ETHICAL AND REGULATORY CONSIDERATIONS

### 16.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

### 16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

### 16.3. Approvals

Approvals will be requested and issued based on the submitted documentation (as per regulatory requirements) from both the Hospitals' Ethics Committees and Central Ethics Committee and from Ministry of Health of Republic of Croatia as the regulatory authorities for the approval of clinical trials.

### 16.4. Other Ethical Considerations

There will be no use of placebo in this trial. Both groups will receive standard treatment and will be followed during the course of disease as necessary according to clinical presentation. There are no banned treatments, and all adverse events would be followed actively. The investigational treatment for this trial will not influence any clinical decisions or other necessary interventions. Other than infants (as vulnerable population per se) as per exclusion criteria (any underlying disease, comorbidity or congenital malformation, children with severe disease as evaluated with the Wang score (>8) and children with progressive respiratory distress requiring mechanical ventilation) children with other vulnerable conditions will not be recruited in this trial. A 2:1 randomization scheme in favour of active treatment will be used.

### 16.5. Reporting

After the approval of Ministry of Health has been given to conduct a clinical study, the applicant is obliged to submit an Annual Progress Report on a yearly basis, by January 31$^{st}$, to the Ministry of Health (MoH) and to the Central Ethics Committee (CEC) on the course of conducting clinical trials in approved site, the total number of subjects in screening and the total number of subjects included in the study.

The applicant is obliged to inform the Ministry of Health and the Central Ethics Committee on any changes and addendums to the clinical trial. Significant amendments require the opinion of the CEC and the approval of the MoH. For minor administrative changes and amendments, only notification to the CEC and the MoH is required.

End of Trial notification has to be submitted to the CEC and the MoH within 90 days from the date of completion of clinical trials. The end of the clinical trial is considered to be the day of the last examination of the last subject involved in the clinical trial.

In case of early termination of the clinical trial or temporary suspension, the applicant is obliged to report it to the CEC and the MoH within 15 days, providing a detailed explanation of the cause of the early termination/suspension.

After the end of the clinical trial, the applicant is obliged to submit the Final report to the CEC and the MoH, within 1 year from the end of the date when the clinical trial has ended.

### 16.6. Participant Confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants‘ personal data.

### 16.7. Expenses and Benefits

Travel expenses related to participants' study visits will be reimbursed with a maximum amount of € 25 per visit. Participants will be reimbursed according to the proof of travel costs for study purposes, which will be given to the study team. Participants will not receive any other compensation.

## 17. FINANCE AND INSURANCE

### 17.1. Funding

The financing of this trial is provided by Sponsor. Details regarding trial's funding and distribution are specified in the Contract between CRO and site.

### 17.2. Insurance

Sponsor will provide insurance for all subjects participating in the clinical trial in line with local applicable laws. The insurance policy details are provided in the Investigator's file and in the Informed Consent Form.

## 18. PUBLICATION POLICY

The publication, presentation, or other public disclosure of study results (each, a "Publication") will be accurate and honest, undertaken with integrity and transparency and in accordance with the Sponsor's approval.

Publications of results will be subjected to fair peer-review. Overall, published results will include the results of the statistical analysis of the study endpoints and will contain the data of all available patients which will comply with the guidelines of the International Committee of Publishers of Scientific Journals (ICMJE). The list of authors for the publication will include the names of participating center researchers in accordance with the principles governing scientific publications. The primary authors of the publication

of the study results will be the Principal Investigators and the Scientific Officer of the Sponsor listed on the signature page of this protocol.

The Investigator will notify the Sponsor in advance of their intention to publish or present study data. Any presentation or publication of all or part of the results (abstracts in journals or newspapers, oral presentations, etc.) by the Investigator or his representatives will require prior submission for review by the Sponsor. Sponsor will not block or prohibit publication(s) but reserves the right to delay a publication in order to protect intellectual property rights, in agreement with the provisions of the contract signed between the Sponsor, the Institution and the Investigator.

## 19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

All the results, data, documents, discoveries and inventions (collectively hereinafter the "Inventions") which arise directly or indirectly from the conduct of the study, shall be the immediate and exclusive property of the Sponsor. The Institution and the Investigator will assign to the Sponsor all intellectual property rights which may arise directly or indirectly from the Study and all existing or future materials created in relation to the Study, in agreement with the provisions of the contract signed between the Sponsor, the Institution and the Investigator.

## 20. ARCHIVING

All clinical trial information shall be recorded and stored by the Sponsor or Investigator, as applicable, in such a way that it can be accurately interpreted and verified while the confidentiality of the personal data of the subjects remain protected. These documents may be maintained in multiple locations, depending on whether they are stored with regulatory files or as participant documents. The Sponsor and the Investigator should maintain a record of the locations of their essential documents.

Full data exported from the eCRF will be shared with the study site after the database lock.

Due to inspection by regulatory authorities, eCRF will be kept for at least 15 years after the completion of the study, while the content of ISF and TMF is archived by the Investigator and the Sponsor, as applicable, for at least 25 years after the end of the clinical trial.

## 21. REFERENCES


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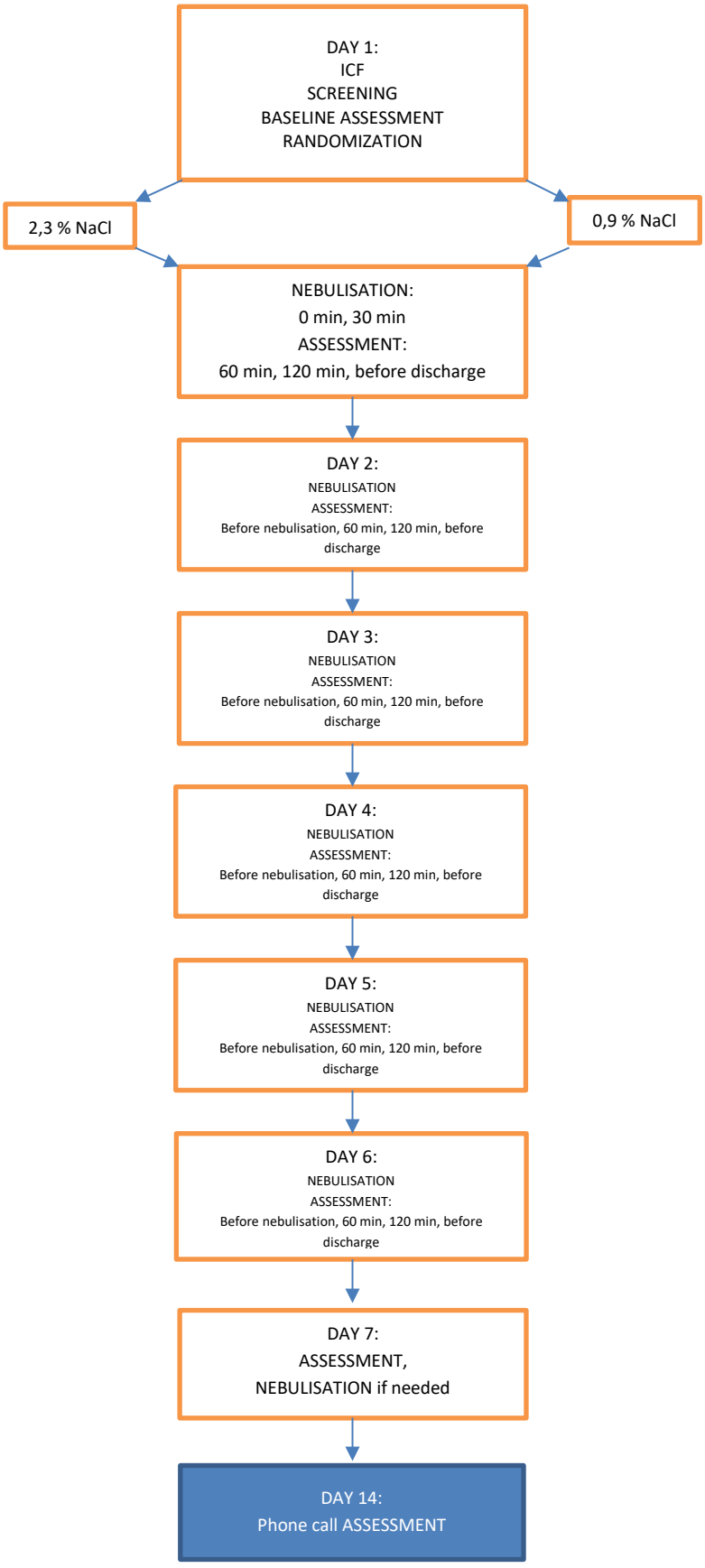
## 22. APPENDIX 1: TRIAL FLOW CHART



## 23. APPENDIX 2: SCHEDULE OF ASSESSMENTS

| | | Treatment Days | | | | | | | Follow up |
|---|---|---|---|---|---|---|---|---|---|
| **ASSESSMENTS** | **SCREENING DAY 1** | **Day 1 (Baseline)** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 7 (End of Study)** | **Day 14** |
| **Informed Consent** | X | | | | | | | | |
| **In-/exclusion criteria** | X | | | | | | | | |
| **Demographics**[1] | X | | | | | | | | |
| **Weight** | X | | | | | | | | |
| **Physical exam. (PE)** | X | X | X | X | X | X | X | X | |
| **Vital signs**[2,4] | X | X | X | X | X | X | X | X | |
| **Wang Score**[3,4] | | X | X | X | X | X | X | X | |
| **$SpO_2$**[4] | | X | X | X | X | X | X | X | |
| **Randomization** | X | | | | | | | | |
| **Discharge or hospital admission evaluation**[5] | | X | X | X | X | X | X | X | |
| **Provision of Ventica device (where applicable)**[6]**, subject diary and nebuliser**[7] | | X | | | | | | | |
| **Return of Ventica device (where applicable)**[6]**, subject diary and nebuliser**[7] | | | | | | | | X | |
| **Measurement of lung function with Ventica device**[8] | | X | | | X | | X | | |
| **Ventica device data download**[9] | | | | | | | | X | |
| **Filling out subject dosing diary at home** | | X | X | X | X | X | X | | |
| **Return of used vials from the previous day** | | | X | X | X | X | X | X | |
| **Persistent cough** | | | | | | | | X | |
| **Total number of unscheduled visits**[10] | | | | | | | | X | |
| **Total number of lost days of work**[10] | | | | | | | | X | |
| **Follow up call**[11] | | | | | | | | | X |
| **Concomitant medication**[12] | | X | X | X | X | X | X | (X) | |
| **Nebulized saline**[13] | | X | X | X | X | X | X | (X) | |
| **Patient compliance**[14] | | ← | — | — | — | — | → | | |
| **AE monitoring** | | ← | — | — | — | — | — | → | |
| **Other intervention**[15] | | ← | — | — | — | — | — | → | |

1. Includes date of birth, age, sex, and race.
2. Vital signs examination includes: evaluation of body temperature, respiratory rate, and heart rate. The physical exam, vital signs, Wang score and $SpO_2$ results on Days 2-7 will determine if any unscheduled hospital visit and hospitalization within the next 24 h between visits is required.
3. Wang score will be determined according to Wang et al, Arch Dis Child. 1992;67:289–93.
4. Evaluation will be conducted at each study day (Days 1-6) prior to any treatment, 60 and 120 min after the first hospital nebulization session (Days 1-6) and before discharge for this day and on Day 7.

5. To be conducted at the end of each evaluation day (Days 1-7). The physical exam, vital signs, Wang score and $SpO_2$ results on Days 2-7 will determine if any unscheduled hospital visit and hospitalization within the next 24 h between visits is required.
6. Ventica device will be optional for patients. If they agree to use it, a device will be provided by the site on Day 1.
7. For the needs of the study, if patients do not have nebulizers, they will be provided by the site on Day 1.
8. Ventica device overnight measurements: Starting Day 1, Day 4 and Day 6 (completed Day 2, Day 5, Day 7, respectively).
9. Ventica device data will be downloaded on Day 7.
10. Corresponds to total numbers for the whole period of the trial since baseline. The physical exam, vital signs, Wang score and $SpO_2$ results on Days 2-7 will determine if any unscheduled hospital visit and hospitalization within the next 24 h between visits is required.
11. An assessment of patient symptoms related to bronchiolitis or AE will be performed by phone.
12. Includes standard medication and any other medication taken by the patient during the study. Standard medication includes nebulized bronchodilator. Hospital administration: Day 1: at 0 and 30 minutes, followed by two home nebulization sessions. Days 2-6: at 0 minutes, followed by two home nebulization sessions. On Day 7: treatment will be provided as needed.
13. Nebulized saline administration (2.3% or 0.9% NaCl). To be administered immediately after nebulized bronchodilator administration. Day 1: immediately after the two hospital nebulization sessions and the two home nebulization sessions. Days 2-6: immediately after the hospital nebulization session and the two home nebulization sessions. On Day 7: treatment will be provided as needed.
14. Recording of appropriate number of home nebulization sessions of standard medication followed by nebulized saline.
15. Recording of any other treatment during the course of the study, such as nose unblocking, oxygen therapy or rehydration.

## 24. APPENDIX 3: PROTOCOL AND AMENDMENT HISTORY

| **Protocol / Amendment No. and date** | **Protocol Version No.** ***(amendment related to version of protocol)*** | **Date issued** | **Details of Changes made** |
|---|---|---|---|
| Protocol v 01 – 08MAR2023 | V01 | 08MAR2023 | ORIGINAL PROTOCOL VERSION |
| Protocol v1.1 – 21Apr2023 | V1.1 | 21Apr2021 | Response to CEC queries accepted and integrated |
| Protocol v2.0 – 27Feb2024 | V2.0 | 27Feb2024 | Prolongation of trial period and inclusion of additional study sites |

## 25. APPENDIX 4: INFORMED CONSENT FORM LOG

| **INFORMED CONSENT FORM (specify title)** | **ICF Version No.** | **Date issued** | **Details of Changes made** |
|---|---|---|---|
| INFORMED CONSENT FOR PARENTS | 01 | 08MAR2023 | ORIGINAL ICF VERSION |
| INFORMED CONSENT FOR PARENTS | 1.1 | 21APR2023 | Corrections requested by CEC incorporated |
| INFORMED CONSENT FOR PARENTS | 2.0 | 27FEB2024 | Prolongation of trial period and inclusion of additional study sites |
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