PROTOCOL SYNOPSIS

Title:	Post-market, confirmatory, interventional, randomized and controlled clinical study to assess the efficacy and safety of Sentinox in COVID-19 patients.
Study center:	Principal investigator: Prof. Giancarlo Icardi Investigational site: Ospedale Policlinico San Martino IRCCS, Largo Rosanna Benzi - 16132 Genova Department: Direttore dell'Unità Operativa di Igiene Tell: 010/555 2375 Email: icardi@unige.it
Study period:	Study approval: Q2 2021 Date of first enrolled patient (FPI): Q2 2021 Date of last completed patient (LPO): Q4 2021 Total enrolment period: 6 months Total study duration: 7 months
Study phase:	Post-Market Study - Medical Device
Study Rationale:	Since December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the beta-coronavirus family, has spread globally, leading to a pandemic of coronavirus disease 2019 (COVID-19). Whereas the COVID-19 has occasioned over 100 million cases and more than 2.3 million deaths worldwide, the published results of pivotal trials of the first COVID-19 candidate vaccines have represented a source of genuine hope for the international community. Numerous countries have rapidly initiated a COVID-19 vaccination campaign; as of 12 February 2021, more than 150 million doses had been administered throughout the world. However, until now, the vaccines are not available for all the people stressing the importance of preventative measures in managing the spread of the virus. Although the majority of COVID-19 cases are considered mild, a subset of patients develops severe respiratory symptoms with high viral load. Similar to other coronaviruses, SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE-2) receptor on epithelial cells. Recently, some authors have quantitated differences in ACE-2 receptor expression and SARS-CoV-2 infectivity in the nose (high) versus the peripheral lung (low). For these and other evidences researchers speculated that nasal surfaces might be the dominant initial site for SARS-CoV-2 respiratory tract infection. Data in COVID-19-positive subjects support the concept of early infection in the upper respiratory tract (0–5 days) followed by subsequent aspiration and infection of the lower lung. Nasal irrigations are a common practice of upper respiratory tract care, used either alone or in association with other therapies in several conditions affecting the upper respiratory tract.

	In this scenario the possibility to reduce the virus load in the nose of already affected COVID-19 patients by nasal washing solution could reduce potential additional virus load, able to contribute to reduce the spreading of viral infection. APR SA has recently obtained the CE Certification as medical device for Sentinox, a solution intended for irrigation, cleansing and moistening of nasal cavities. However, until now, no data from clinical trial are available about the use of Sentinox in COVID-19 patients. For these reasons, a post-market, confirmatory, interventional, randomized and controlled study was planned to evaluate the safety and performance of Sentinox in mild COVID-19 patients, as an addon treatment to standard therapy.
Study Objective:	**Primary** • To evaluate the performance of Sentinox intranasal administration in mild COVID-19 patients **Secondary** • To evaluate the change of clinical features of disease • To evaluate the tolerability and safety of Sentinox • To evaluate the patient satisfaction of the Sentinox.
Methodology:	This is a post-market, confirmatory, interventional, randomized and controlled study to assess the efficacy and safety of Sentinox medical device in the treatment of mild COVID-19 patients (defined according WHO guideline, version of 27 May 2020). The study will consist on 9 visits. At screening visit, according the investigational site procedures, patients with a positive COVID-19 nasopharyngeal swab (quantitative swab test with RT-PCR Ct value $\leq$ 30 for at least 2 genes out of 4) performed at investigational site in the same day will be summoned. Patients will be enrolled after having signed the informed consent form prior to any other study procedure and after inclusion/exclusion criteria check. According the investigator judgment, the patient's clinical outcomes and the investigational site guidelines, the enrolled patients should be hospitalized or redirected to other structures (e.g. "COVID-19 hotel", patient's home). At each visit, all study procedures will be performed according to the clinical investigation plan requirements (see flow-chart). At Visit 0, the patient will be randomized with a 1:1:1 ratio in one of 3 trial groups: - GROUP A: IP treatment performed 3 times/day for 5 days (as addon to the standard therapy) at 8am, 2pm and 8pm;

	- GROUP B: IP treatment performed 5 times/day for 5 days (as addon to the standard therapy) at 8am, 11am, 2pm, 5pm and 8pm; - GROUP C: no IP treatment; only the standard therapy will be performed. At same Visit (Visit 0) the enrolled patients will be trained to self-administer IP treatment according the assigned trial group. To ensure the treatment compliance, at each treatment day (from Visit 1 to Visit 5), 10 minutes before each planned administration, the investigational staff will remind the patients to perform the IP treatment according to assigned group (e.g. by phone contact for patients redirected to other structures). At Visit 1 and Visit 2, three nasopharyngeal swabs will be performed at 8:40 am ± 10 min, 2:40pm ± 10 min and 8:40pm ± 10 min. At subsequent planned visits only one nasopharyngeal swab will be performed at 8:40 am ± 10 min. The patients should blow the nose before performing each nasopharyngeal swab. For patients redirected to other structures above mentioned, investigational staff will reach the patients to perform the indicated trial procedures. From Visit 1 to Visit 5, patients will record daily Adverse Event (AE), concomitant medication and presence of clinical features COVID-19 related (nasal congestion, fever, dry cough, wet cough, difficulty breathing or shortness of breath, loss of taste, loss of smell, tiredness, muscle discomfort/pain, sore throat, diarrhea/vomiting, conjunctivitis, headache, skin rash/discoloration of toes, chest pain/pressure) in a diary. The subject will be instructed how to complete the diary at Visit 0. In the diary will be reported an illustrative representation of IP' self-administration procedure too. Principal Investigator and the involved collaborators will give high priority to the health and the well-being of each enrolled patient until the end of the study. According the investigator judgment, the necessity to treat the patient with antiviral therapy or the worsening of clinical condition (defined as per WHO guideline above mentioned) will be considered reasons for patient withdraw.	
Number of subjects (planned and analysed):	Planned:	57 patients in total (19 per group). The design of the trial is to test the superiority of Sentinox treatment versus Standard treatment in the reduction of viral load (copies/mL) in mild COVID-19 patients nasal fluids at any time, during the five days of treatment. Data published by Chu, Zou and Han [Mi Seon Han et al., Lancet 2020, 21:165; Lirong Zou et al., N Engl Med 2020, 382:1177-79; Daniel K.W. Chu et al, Clinical Chemistry 2020, 66:549-555] have been used to infer on the basal viral load in the reference population. Assumptions used in the sample size calculation are:

		- Delta between treatments: at least 1.5 Log_{10} (copies/mL) - Standard Deviation: 1.2 Log_{10}; it has been assumed from publish data around 80% of the reference mean difference - Probability threshold: 0.025, considering that Sentinox has two different treatment schedule (3 and 5 time die) Bonferroni correction on probability level has been applied (Sentinox1 vs Standard Treatment; Sentinox2 vs Standard Treatment) - Power: 80% 14 Patients in each treatment groups will be enough to detect at least 1.5 Log_{10} difference between treatments at any time post treatment. Applying a drop-out correction of 25%, 19 patients in each group will be randomized.
	Treatment duration:	Patients will be randomized in one of treatment arms: - GROUP A: IP treatment performed 3 times/day for 5 days (as addon to the standard therapy); - GROUP B: IP treatment performed 5 times/day for 5 days (as addon to the standard therapy); - GROUP C: no IP treatment; only the standard therapy will be performed.
	Safety Analysis Set (SAS):	This is the subset of subjects who are randomized and consumed at least one dose of study product regardless of any protocol deviations.
	Intention to Treat Analysis Set (ITT):	This is the subset of subjects who are randomized and consumed at least one dose of study product and who has available data for the variables of interest.
	Per Protocol Set (PPS):	This is the subset of subjects who are randomized, has consumed at least one dose of study product, who has available data for the variables of interest and who has no major protocol deviations.
Diagnosis and main criteria for inclusion:	Inclusion criteria:	• Patient Informed consent form (ICF) signed; • M & F Aged ≥ 18 years and ≤ 64 years at the time of the signature of ICF; • Subjects who are willing to comply with the requirements of the study protocol, attend scheduled visits and calls for the duration of the study by telephone contact; • Mild Symptomatic Individuals with COVID-19 confirmed by polymerase chain reaction (PCR)

		based on WHO guideline (version of 27 May 2020). In the study COVID-19 patient with RT-PCR Ct value ≤ 30 for at least 2 genes out of 4, at the first swab, will be enrolled. The enrollment of COVID-19 vaccinated patients will be allowed if they will present a *"clinical vaccination failure"*, defined according to the indications reported in the "Global Manual on Surveillance of AE Following Immunization" (WHO guidelines). • Onset of symptoms from not more than 2/3 days
	Exclusion criteria:	• Other clinically significant and uncontrolled pathologies that may interfere with study results (e.g. rheumatic diseases); • Presence of any relevant organic, systemic or metabolic disease (particularly significant history of cardiac, renal, neurological, psychiatric, oncology, endocrinology, metabolic or hepatic disease), or abnormal laboratory values that will be deemed clinically significant based on predefined values; • Immune system illnesses; • Known drug and/or alcohol abuse; • Individuals who are cognitively impaired and/or who are unable to give informed consent; • Ongoing or prior participation in any other clinical trial of an experimental treatment for COVID-19 ; • Ongoing or prior participation in any other clinical trial of an experimental treatment within 30 days from enrollment day; • Intubated or prior intubation (during present hospitalization) or anticipated intubation within the subsequent 2 hours; • Using high-flow nasal cannula (HFNC) or non-invasive ventilation (NIV); • Concurrent or planned treatment with other agents with actual or possible direct antiviral activity;

		• Prior hospitalization for COVID-19; • Positive pregnancy test or breastfeeding woman; • Known hypersensitivity to the study treatment, its metabolites, or formulation excipient; • History of severe drug and / or food allergies and / or known allergies to the trial product or its components; • Any condition that, in the opinion of the Investigator, would complicate or compromise the study or well-being of the patient.
Investigational Product:	Study product:	Sentinox is an AcidOxidizing Solution (AOS), available as 50ml bottle, intended for irrigation, cleansing and moistening of nasal cavities. The device is indicated for: - reducing the risk of infections caused by bacteria and viruses, including SARS-CoV-2 (etiologic agent at the basis of the pandemic COVID-19, as supported by in vitro test), by lowering the nasal microbial load; - symptomatic nasal care (to ease nasal symptoms, e.g. nasal congestion, typical of upper respiratory conditions such as cold, flu, sinusitis or rhinitis); - nasal care in case of minor lesions/alteration of the nasal mucosa (e.g. creates an ideal environment for an optimized resolution of irritation and lesions). Sentinox contains hypochlorous acid (HClO), a known antimicrobial agent acting as a preservative by inhibiting the growth of microorganisms within the solution, and exerting an ancillary local antimicrobial effect on the nasal mucosa.
	Dose/dosage:	5 sprays into each nostril repeated 3 or 5 times according the assigned trial group (i.e. GROUP A or GROUP B respectively; for GROUP C no IP treatment is planned).
	Administration:	The application of IP on should be performed in accordance to the following indication: The treatment is administered in a dose of 0.5 ml into each nostril (5 sprays) per the number of times per day according the trial group assigned according the randomization procedure (i.e., GROUP A: 3 times/day; GROUP B: 5 times/day; GROUP C: no IP treatment). The instruction for the patients will be:

		- Tilt slightly the head backwards. - Close one nostril and gently insert the nozzle into the other. - Gently squeeze the pump 5 times as you breathe in. - Switch to the other nostril and repeat. - Wait at least 1 minute and then blow the nose. At Visit 0 the enrolled patients will be trained to self-administer IP treatment according the assigned trial groups. In the diary dispensed at Visit 0 will be reported a graphic simplification of self-administration procedure too.
Criteria for evaluation:		
Primary endpoint	To assess the efficacy of Sentinox versus Standard Treatment in term of reduction in viral load (copies/mL) in nasal fluids in mild COVID-19 patients	
Secondary endpoints	• To assess the efficacy of Sentinox versus Standard Treatment in term of reduction in viral load (copies/mL) in nasal fluids in mild COVID-19 patients stratifying the results according to the initial viral load (i.e. "high viral load" is defined as RT-PCR Ct value < 20; "medium viral load" is defined as RT-PCR Ct value between 20 and 30) • To evaluate the time profile of Sentinox to affect the profile of viral load (copies/mL) analyzing the subjects' negativization (expressed as number of negativized patients; negative status is defined as "1 or 0 detectable gene" by RT-PCR); • To evaluate the time profile of Sentinox to affect the profile of viral load (copies/mL) analyzing the infectiousness of the patients (expressed as number of infective patients; not infectious status is defined using the cycle threshold value of >35 cycles); • To compare the two treatments schedule of Sentinox: 3 irrigations/die versus 5 irrigation/die • To evaluate the time profile of Sentinox to affect the profile of viral load (copies/mL) during the duration of the study (treatment period and follow-up period); • To evaluate the time profile of Sentinox to affect the duration of clinical features of disease using an ad hoc questionnaire during all study duration; • To evaluate the tolerability of the Sentinox a Visual Analogue Scale (VAS) will be used; • The patient satisfaction will be evaluated with a 5-points Likert Scale.	
Safety:	Safety will be monitored through clinical examination and Adverse Events including assessment of relationship to the IP.	
Time-points for efficacy and safety:	All planned visits.	

Interim Analysis:	If a slowdown in enrolment of patients will occur, an interim analysis will be performed considering the data from the first 30 enrolled patients (10 patients for each treatment GROUP) who completed Visit 8 (EOS). The safety and efficacy analysis will be performed on all defined endpoints according to the procedures described in the statistical methods section. The purpose of this analysis is to verify and to confirm the hypotheses formulated in the sample size, taking into account that vaccinated patients, clinically failed in the vaccination, will be also enrolled in the study. Inferential analysis on the primary endpoint will be done using ANCOVA in which baseline value of viral load and non-vaccinated/vaccinated variable will be the co-variate.
Statistical methods:	In general, all the variables will be descriptively analyzed by treatment groups and visit (mean, median, standard deviation, minimum and maximum for continuous variables after normality check of distribution with Kolmogorov-Smirnov test, frequency distribution for categorical variables). All the analysis will be detailed in the Statistical Analysis Plan (SAP) which will be finalized in Version 1.0 before the Data Base Lock (DBL). In details, the safety data will include (at least) patient examinations, laboratory data and adverse events.