INCIPIT- IN*haled liposomal* C*yclosporine* A *for COVID-19* P*neumon*IT*is*

Title: A proof-of-concept study of the use of Inhaled liposomal -Cyclosporin-A in the treatment of moderate COVID-19-related pneumonia: a two-step phase II clinical trial

Background: Since December 2019, there has been an outbreak of Coronavirus Disease 2019 (COVID-19) responsible for a severe acute respiratory syndrome, often requiring hospital admission for ventilatory and medical support [1]. The regions with the highest prevalence are Veneto, Emilia Romagna, Piedmont and Lombardy. According to data released by our National Institute of Health (Istituto Superiore di Sanità - ISS) on April 28.2020 the incidence of COVID-19 infection in these regions (according to the number of reported symptomatic cases) was respectively between 361 and 739 patients/100.000 inhabitants, with a total of 142.232 confirmed cases, 74.346 in Lombardy. According to the integrated surveillance system report among reported symptomatic cases, 21.3% manifested a severe disease, and 3% needed Intensive cares [2]. There are no currently approved treatments for COVID-19 infection. Treatment with anti-viral agents combined with hydroxychloroquine is based on the available preclinical evidence and clinical experience collected since the infection outbreak and on experimental data on the 2003 SARS epidemic [3,4]. Despite these treatment measures, worsening of gas exchange and development of an acute respiratory distress syndrome (ARDS) requiring continuous positive airway pressure therapy or mechanical ventilation with admission to the intensive care units (ICU) are relatively common [1].

Although formal definitions have been proposed for stratification, no consensus exists to date [5]. For this document, patients with COVID-19 will be stratified into mild, moderate, and severe disease based on clinical triage as follows:

- Mild: Mild symptoms, no shortness of breath or hypoxia
- Moderate: Shortness of breath or hypoxia requiring supplemental oxygen via nasal cannula
- Severe: Respiratory failure requiring intensive care unit admission. Need for ventilatory support, acute respiratory distress syndrome, circulatory collapse, acute kidney failure, cardiomyopathy, and/ or clinical syndrome compatible with cytokine storm.

There is accumulating evidence that the infection has a "multi-step" course corresponding to different phases of the innate and specific antiviral response activation [6]. The viral load in nasopharyngeal swab has been shown to decrease over time and become negative between illness day 9 and 14 in four patients

recovering from pneumonia, while in most severely ill patients, nasopharyngeal virus detection persisted until death [7]. Specific IgM has been reported to peak at day 9 in plasma of an infected patient, while IgG starts to increase at day 9 and peak at day 19-20 [6]. Another study assessed by single-cell sequencing analysis the innate immunity activation profile in early versus late recovery from COVID-19 pneumonia. In an early stage, authors detected an increase in the ratio of classical CD14+ IL1b monocytes and a lower ratio of T and NK cells but an increase in the proportion of T cells expressing inflammatory genes, at a high rate (JUN, FOS, JUNB, and KLF6). In this early phase, B cell-derived IL-6, T cell-derived CSF1 (M-CSF), and CSF2 (GM-CSF) may promote monocyte proliferation and activation. As a result, monocytes may produce a larger number of inflammatory mediators, including IL-1β and IL-6, contributing to an inflammatory storm. On the contrary during a late recovery phase patients had an increase in T and NK cells in the periphery, with a lower expression of inflammatory genes, DCs-derived TNFSF13 and IL-18 and T cell-derived IL-2, IL-4 were suggested to promote B cell survival, proliferation, and differentiation [8]. Thus a more robust Th-induced monocyte-macrophage activation is thought to be associated to the more severe outcome, in fact, it has been demonstrated that Patients needing ICU care had higher plasma levels of many innate cytokines (IP-10, MCP-1, MIP-1A, IL6, IL8, IL10 and TNFα), lower peripheral lymphocytes counts and higher neutrophil counts [9,10]. Thus, it is current belief that CD4+ T-helper lymphocytes undergo a coronavirus - induced huge activation with a Th1 profile, produce a high amount of GM-CSF thus activating macrophages and consequent high release of proinflammatory cytokines. This macrophage activation pattern is present at the lung level, as demonstrated recently analyzing Bronchoalveolar lavage of COVID 19 pneumonia patients (3 with severe and 3 with a mild disease. These Authors demonstrated that monocyte-derived FCN1+ macrophages (highly inflammatory and enormous chemokine producers such as CCL2, CCL3, CCL5, IL-8, CXCL9, CXCL10 and CXCL11) which infiltrated the lung, represent a predominant macrophage subset in BALF from severe ARDS patients, thus identifying the FCN1+ macrophages as the culprit for the deranged hyper-inflammation [11].

Rationale:

Potential new candidate treatments for COVID-19 acute respiratory disease have been identified using machine learning techniques by connecting medical information regarding therapies that could block the viral infection replication and contain the exaggerated immune response. Among them, several immunosuppressants with different mechanisms of action, from direct anti-cytokine blockade as new anti IL6 and IL6R blockers, anti-IL1R to a more pleiotropic Janus kinase (Jak) inhibition with the targeting of Jak1-2 or Jak1-3 have started to be explored or proposed [12–16].

Cyclosporin A (CsA) deserves a particular interest in this context because it combines a well-known immunosuppressive activity with a pan-antiviral activity. CsA immunosuppressive action is driven by the inhibition of T-cells and macrophages and is mediated by the blockade of calcineurin and consequently of the nuclear translocation of the Nuclear Factor of Activated T Cells (NF-AT). CsA also exerts direct antiviral and anti-inflammatory activity through the inhibition of the peptidyl-prolyl cis-trans isomerase activity associated with the family of cyclophilins (Cyps). In particular, CypA inhibition by CsA can block the replication

of human and animal coronavirus strains, such as SARS-CoV and MERS-CoV, likely preventing correct folding of proteins indispensable for viral replication [17–19]. Moreover, CsA significantly decreases the CD147-dependent chemotaxis activity of CypA that induce migration of leukocytes to the sites of inflammation in mouse models of acute lung injury, asthma, and rheumatoid arthritis [20–22]. Interestingly, some Coronavirus strains can stimulate NF-A, thus activating T-cells [23]. Furthermore, SARS- and MERS-CoV have been shown to down-modulate effective antiviral immunity and enhanced pro-inflammatory cytokines, by several mechanisms. In fact, SARS-CoV and MERS-CoV are equipped with a mechanism that inhibits type I IFN signalling, decreasing STAT1/2 phosphorylation and inhibiting nuclear translocation of STAT1 [24]. MERS coronavirus infection is also associated to an upregulation of C-type Lectin Receptors and of retinoic acid I inducible like receptors significantly contributing to macrophage activation, pro-inflammatory cytokine and chemokine production (among which CXCL1), and ultimately neutrophil activation and Net-osis which participates to severe tissue injury [25]. With these premises, it is reasonable to assess whether CsA has efficacy in the treatment of COVID-19 pneumonia.

An interesting strategy to improve the lung delivery of CsA is represented by its incorporation into the bilayer of liposomes. To this aim, a solution of FDA approved liposomes (L-CsA) diluted in sterile NaCl has been developed for an inhalation administration through a certified nebuliser ((PARI Investigational eFlow Device (L-CsA eFlow). *In vitro* studies have already shown a significant inhibitory effect of L-CsA on the growth of a broad range of microbes (Candida albicans, Aspergillus niger, Aspergillus fumigates and their combination) [26]

Protocol Code:

EudraCT Number:

Phase: Phase II

Test product: Liposomal cyclosporin-A (L-CsA). The L-CsA is supplied in glass vials containing 10 mg of L-CsA. The 10 mg L-CsA lyophilisate is reconstituted with 2.4 mL 0.25% NaCl to obtain a ready to use dispersion for inhalation via the PARI Investigational eFlow Device (L-CsA eFlow) registered EU with the following number EU (001947134).

Control product/placebo: SOC, per standard institutional practice

Dosage: Inhalation of 10mg L-CsA with PARI Investigational eFlow Device will be of 8-13 Min.

Treatment Duration: Inhalation of L-CsA with PARI Investigational eFlow Device (L-CsA eFlow) will be performed twice daily for 7 days.

Objectives: The objective of the study is to assess the safety profile and preliminary efficacy of Inhaled LCsA in the treatment of patients with COVID-19 pneumonia characterized by a moderate disease and a high inflammatory status (CRP>5mg/dl, IL6 levels>40 pg/ml) anticipating a high risk of progression to severe ARDS.

Primary Objective

The primary objectives will be the safety and a proof of concept of efficacy of the treatment.

Secondary Objectives

Secondary objective will be the quantification of the efficacy and safety parameters.

Design: This will be a two-step trial.

There will be safety phase aimed at assessing safety profile of Inhaled L-CsA in a limited number of patients (n = 5) with the acute respiratory syndrome (SARS)-CoV-2 pneumonia accompanied by signs of high inflammatory status. (PaO2/FiO2 300-200 in Fi=2</=60%). Enrolled patients must be able to perform approximately 15 min aerosolization. L-CsA will be administered as illustrated below (study procedures).

If the initial safety phase will lead to favourable results (at least 80% of patients (4/5) tolerate well the treatment without major adverse events - the need of suspension of inhalatory treatment NOT related to drug toxicity but to inability to perform saline inhalation will not be considered major adverse event), an open-label, phase II randomized controlled pilot trial to assess the initial efficacy will be conducted. Patients included in the safety Phase will not be part of the efficacy cohorts. The L-CsA arm patients will be eligible if they satisfy the eligibility criteria.

Patients parallel groups will be allocated in a 1:1 ratio to assess the hypothesis of potential superiority of L-CsA in adjunction to current standard antiviral and supportive therapy, as compared to antiviral and supportive therapy alone. Given the emergency situation that is being faced with COVID-19 an open-label design has been chosen to accelerate the realization of the study.

The first phase of the study is a safety step, the use of randomization and objective measure of the response will minimize bias in the efficacy exploratory study.

Sample Size: Safety phase (n=5)

Efficacy phase (n=26), 13 pts per arm

Dropouts are not expected. In case they would occur, they will be replaced by an additional patient.

The primary outcome of this study is represented by safety of Inhaled L-CsA. This is why the study has a first Safety Phase whose primary outcome needs to be met in order to proceed with the second phase. The second phase will explore the efficacy of LCsA inhalation in COVID-19 pneumonia with a pilot study designed according to Cocks design [32].

For a future large study, we hypothesize a difference between a proportion of success 40% in the control arm and of 65% in the treatment arm to be clinically relevant. This hypothesis is based on the personal experience of the PI. This would require to enroll 124 patients (62 per group), when the power is 80% and the type I error is 5%.

An external pilot study of an overall trial designed with a power 80% and a type I error 5%, would aim at showing whether the treatment estimate is larger than zero. Using the one sided-90% confidence interval approach, with 26 patients (13 per arm), the lower 90% confidence limit for a zero difference would be 24.4%, excluding the 25% treatment effect estimate. In this case the pilot study would point toward the presence of a treatment effect.

Population:

The following definitions have been used to better identify the study population:

1) Confirmed SARS-CoV-2
   Defined as the identification of unique sequences of virus RNA by nucleic acid amplification tests, such as real-time reverse transcription polymerase chain reaction (RT-PCR) on respiratory specimens.
2) Acute Respiratory Distress Syndrome
   The Berlin Definition (30) will be used to define moderate and severe oxygenation impairment as follows:
   - Mild: 200 mm Hg < PaO2/FIO2 $\leq$ 300 mm Hg
   - Moderate: 100 mmHg < PaO2/FiO2 $\leq$ 200 mmHg
   - Severe: PaO2/FiO2 $\leq$ 100 mmHg

Patients fulfilling all of the following inclusion criteria are eligible for the study

Inclusion criteria

- Ability to obtain informed patient consent noting the limitations of existing knowledge regarding Inhaled Liposomal CSA efficacy and the labeled warning and precautions as the proposed use is outside the approved indication.
- Informed Consent as documented by signature
- Adult patients aged 18-74 years-old
- Bilateral infiltrates at chest radiography
- $\geq$48 hours from admission
- Positive Nasal Swab at PCR assay specific for SARS-CoV-2
- Moderate acute respiratory syndrome (SARS)-CoV-2 pneumonia PaO2/FiO2 between 300-200 in FiO2 </=60%)
- Ability to perform 8-13 min aerosolization

- Evidence of clear hyperinflammatory state (CRP >5mg/dl, IL6 levels >40 pg/ml)
- Exclusion of coinfection by PTCI < than 0.5 ng/ml

The presence of any one of the following exclusion criteria will lead to exclusion of the participant.

Exclusion criteria:

- Patients aged < 18 years old and ≥ 75 years old
- Concomitant and documented bacterial infection or PCTI > 0.5 ng/ml
- Lymphopenia less than 200/mmc
- Hemoglobin < 8 g/dl
- Absolute neutrophil count < 1 x 109 cells/L
- PaO2/FiO2 not included in the following interval 300-200 need of a FiO2 support > than 60%
- Treatment with CPAP and /or inability to perform a 10 min aerosolization course
- Sudden clinical deterioration requiring ICU access or palliative care
- Known hypersensitivity or allergy to CSA
- Creatinine clearance < 30 mL/min;
- Severe hepatic impairment
- Pregnant or breast-feeding
- Active tuberculosis
- Evidence of active HBV (HbsAg positive) or with detectable HCV-RNA, HIV

Recruitment and Screening

Patients will be identified and recruited by the treating physician at the COVID-19 wards of the IRCCS Policlinico S. Matteo Foundation of Pavia, Italy. The study will be conducted in the setting of an academic hospital.

Randomization

Patients will be randomized 1:1 to SOC using a computer-generated list built using randomly permuted blocks of varying size. The randomisation list will be generated in Stata 16 (StataCorp, College Station, TX, USA) by a statistician not involved in the study.
Randomization will be performed via web using REDCap (Vanderbilt University, USA).

Criteria for withdrawal/discontinuation of participants

L-CSA will be withdrawn in case of:

- Decrease of PaO2/FIO2 ratio > 40% with respect to basal ratio value
- Need to increase PEEP support /perform CPAP/ start invasive ventilation,
- Onset of bacterial/fungal respiratory infection

- Admission to ICU
- Onset of incapability to perform Inhalatory treatment
- Arousal of any other significant safety issues or drug toxicity sign.

No dose modification is planned in other instances

Dropouts are not expected. In case they would occur, they will be replaced by an additional patient.

End Points:

Primary Outcome:

The primary outcomes will be safety and response to treatment. A patient is considered responder in the absence of oxygenation impairment (>than 40% decrease in PaO2/FiO2 ratio) within 7 days from enrolment.

Secondary Outcomes:

The secondary outcomes associated with the efficacy will be:

- Survival rate within 8 and 28 days
- > than 40% decrease in PaO2/FiO2 ratio
- Peripheral capillary oxygen saturation (SpO2) at day 1,2,3,4,5,6,7,14 and 28
- PaO2 at day 1,2,4,6,7 and 10
- Admission to the ICU
- Length of hospital stay
- Re-admission within 28 days
- The cumulative incidence and severity of adverse events

Monitoring the oxygenation status of the patient with the PaO2/FiO2 ratio will be applied to quantify the rate of moderate or severe oxygenation impairment within 8 days. This will require BGA to obtain the PaO2 value. SpO2 will be monitored over the whole duration of the study. The length of hospital stay will be assessed by the number of days from admission to discharge of the patient or death. Re-admission for complications or relapses of the disease will be assessed up to day 28.

Exploratory Outcomes:

Serial serum samples analyzed for cytokines and biomarkers (including IL-6) to assess the correlation between reduced cytokine/inflammatory mediators, variation in the levels of serum cyclophillins and anti-viral effects of L-CsA treatment according to clinical response.

Safety Outcomes:

Safety outcome will be related to the lack of adverse events. To this aim, the drug-related reactions will be assessed throughout the study. The occurrence of severe infections and herpes zoster reactivation will be recorded.

Significant laboratory abnormalities will be assessed:

- To quantify the rate of each of moderate or severe oxygenation impairment within 8 days
- To quantify the mortality within 7 days
- Peripheral capillary oxygen saturation (SpO2)
- PaO2/FiO2
- To assess the rate of patients admitted to the ICU
- To measure the length of hospital stay
- To quantify 28-day mortality
- To quantify the rate of re-admission within 28 days
- To quantify the cumulative incidence and severity of adverse events

Statistical Analysis:

Primary Analysis:

The rate of response computed as the number of responders over the number of valid patients enrolled (ITT population) will be computed together with its exact binomial 95% confidence interval (95%CI) for each treatment arm. The difference in proportions and 90% confidence interval will be computed.

The response rate will be assessed at day 8, after having completed the full course of L-CsA treatment. This will be the standard duration of antiviral treatment and is in line with the typical duration of the acute disease phase based on clinical experience available to date.

Secondary Analyses:

The rate of each component of the secondary outcomes associated with the efficacy will be:

- The ratio of the number of patients with > than 40% decrease in PaO2/FiO2 ratio will be computed over the number patients enrolled, together with its 95%CI
- The mortality within 8 days will be computed as the number of patients dying over the number patients enrolled, together with its 95%CI
- SpO2 will be assessed with the median and 25th-75th percentiles
- The rate of patients admitted to the ICU will be computed as the number of patients over the number of patients enrolled, together with its 95%CI
- The length of hospital stay will be described with the median and 25th-75th percentiles
- The 28-day mortality will be computed as the number of patients dying over the person time, together with its 95%CI. Overall survival will be plotted using the Kaplan Meier Curve
- The rate of re-admission within 28 days will be computed as the number of patients readmitted over the number patients enrolled, together with its 95%CI
- The number, type, and severity of adverse events will be tabulated. The cumulative incidence and its Poisson 95%CI will be reported.

Interim Analyses:

No interim analyses for efficacy will be performed.

An Interim analysis of the first safety phase of the study will be mandatory (first 5 patients): rate of toxicity, defined by new and worsening adverse events suspected to be correlated with the investigational treatment, will be assessed and examined by a data safety monitoring board (DSMB) of clinicians who are not involved in the study who might advice for the interruption of the study based on safety reasons, if more than 1/5 patients will manifest severe toxicity.

Toxicity leading to the need to discontinue L-CSA has been defined in the intervention section (decrease of PaO2/FIO2 ratio > 40% with respect to basal ratio value; need to increase PEEP support /perform CPAP/ start invasive ventilation; onset of bacterial/fungal respiratory infection; Admission to ICU; onset of incapability to perform Inhalatory treatment; arousal of any other significant safety issues or drug toxicity sign).

Safety Analysis:

Patients will be monitored for treatment- or disease related-AE throughout the duration of the study. Particular attention will be given to L-CSA well-known AE, including the occurrence of infections (including herpes zoster or other serious infections) or laboratory abnormalities.

Computations will be performed with the software Stata 16 (StataCorp, College Station, TX, USA). Patients clinical characteristics will be summarized with the mean and standard deviation or the median and 25th-75th percentiles if continuous and as counts and percent if categorical.

Concomitant Treatments:

Trial Specific Preventive Measures:

Concomitant treatments allowed: antibiotic (any choice), antiviral (according to local protocol), oxygen support (low and high flow until requiring C-PAP or mechanical ventilation), appropriate anticoagulant therapy in patients with risk factors for venous thromboembolism.

Treatments not allowed: palliative care, any kind of concomitant experimental treatment, any other immunomodulatory agent and/or drug agent targeting cytokines or molecular receptors.

Concomitant Interventions (treatments):

The following treatments will be permitted during the trial.

Standard antiviral and antibiotic treatment: according to local protocol (might be modified according to scientific evidences).

All patients with confirmed SARS-CoV-2 pneumonia will start:

Antibiotic therapy consisting of a combination of Azythromycin 500 mg daily or alternatively Ceftriaxone 2 gr/daily or Piperacillin/tazobactam (TZP) full dose according to GFR+doxycycline 200 mg /daily. In case of penicillin allergy, Levofloxacin should be considered as alternative antibiotic therapy.

Oxygen support Low (cannula and simple masks) and high flow (Venturi and reservoir masks, HFNC) will be provided according to the level of hypoxia given a flow of at least 30 lt/min.

Low molecular weight fractionated heparin will be provided according to risk of thrombo-embolism.

If high inflammatory status methylprednisolone 1 mg/kg/day per 5 days, then 0.5 mg/day per other 5 days.

Reporting Safety Information:

Adverse events and drug or device-related reactions will be assessed throughout the duration of the study since the signature of the informed consent to the study by the patient. The occurrence of serious infections and herpes zoster reactivation will be diagnosed clinically or through microbiological tests and recorded. Significant laboratory abnormalities will be assessed. This will include monitoring (every 48 hours or in case of clinical conditions deterioration) full blood count, biochemistry (including liver transaminases), renal function through creatinine values and glomerular filtration rate.

In case of adverse events, information regarding the time of onset, duration, resolution, action to be taken, assessment of intensity, relationship with the study treatment will be recorded.

Assessment in participants who prematurely stop the study

Participants who will prematurely withdraw from the study will be followed to assess and record any adverse event related to the study that might occur during follow-up until day 28.

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Definition of AEs:

The definition of AE and severity is specified below and follows the WHO consensus:

- AE: any untoward medical occurrence in a subject to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment
- Adverse Drug Reaction (ADR): “a response to a drug which is noxious and unintended, and which occurs at doses normally used in man”. The phenomenon is to be intended as noxious (an unexpected therapeutic response, may be a side effect but not an adverse reaction)
- Serious Adverse Event (SAE): any untoward medical occurrence that at any dose requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, results in a congenital anomaly or birth defect, is life-threatening, or results in death
- Unexpected Serious Adverse Event: serious adverse reaction, the nature, severity or outcome of which is not consistent with the reference drug safety information

- Unexpected Adverse Event: an event, the nature or severity of which is not consistent with applicable product information
- A treatment-emergent AE will be defined as any AE that begins on or after the randomization date up to the date of last dose of the study drug plus 30 days.

Summaries of treatment-emergent AE (TEAEs) will be provided for the treatment group.

For the purposes of this study, clinical and laboratory AE will be classified according to the Medical Dictionary for Regulatory Activities (MedDRA) by System Organ Class (SOC), High level Group Term (HLGT), High level term (HLT), Preferred term (PT), Lowest level term (LLT).

Safety reporting:

Reporting of AEs and SAEs during the study will be performed using the MedDRA dictionary V23.

Reporting of AE and SAE:

The investigator will record AEs and laboratory abnormalities according to the protocol as critical to the safety assessment and will report them to Zambon in accordance with the reporting requirements. SAE will be reported within 24 hours from AE identification and acknowledgment. The investigator will record and document all AEs, unless differently specified in the protocol. Where relevant, the investigator will provide a follow-up report to determine whether the SAE has an impact on the benefit-risk ratio of the clinical trial. Zambon will keep detailed records of all AE communicated by the investigator. In case of SAEs occurring after the end of the trial, but with suspected causal relationship to the investigational drug, the investigator will, without undue delay, report the AE to Zambon.

Exceptions from SAE reporting:

Hospitalization for regular therapy as stated in the clinical trial plan. It is assumed that patients will be hospitalized at least from day -2 to day + 7.

Regular follow-up of instrumental or biochemical tests.

Reporting of Suspected Unexpected Serious Adverse Reactions:

The sponsor should report to the Eudra vigilance database, without delay, all relevant information related to suspected SAE, including all suspected unexpected SAE to the investigational drug product occurring in the clinical trial; all suspected unexpected serious adverse reaction related to the drug product occurring in any subject of the clinical trial, even after the end of the trial.

For regulatory reporting purposes Serious Adverse Events will be considered Serious Adverse Reactions when assessed as Related, Probably or Possibly related by either the Investigator.

The period for the reporting of suspected unexpected serious adverse reaction (SUSAR) by the sponsor to the Agency shall take account of the seriousness of the reaction and shall be as follows:

(a) in the case of fatal or life-threatening suspected unexpected serious adverse reactions, as soon as possible

and in any event no later than seven days after becoming aware of the reaction;

(b) in the case of non-fatal or non-life-threatening suspected unexpected serious adverse reaction, no later than 15 days after becoming aware of the reaction;

(c) in the case of a suspected unexpected SAE which was initially considered to be nonfatal

or non- life threatening but which turns out to be fatal or life-threatening, as soon as possible and in any

event not later than seven days after the sponsor became aware of the reaction being fatal or life-threatening.

Where necessary to ensure timely reporting, the sponsor may submit an initial incomplete report followed up by a complete report.

Adverse Events and Causality:

Medication errors, pregnancy and uses outside what is specified in the protocol, including misuse and abuse of the product, shall be subject to the same obligation to report as adverse reactions.

In determining whether an AE is to be considered as an adverse reaction, consideration shall be given to whether there is a reasonable possibility of establishing a causal relationship between the event and the investigational medicinal product based on an analysis of available evidence. Information on causality should be provided by the investigator when reporting to the sponsor. The causality assessment given by the investigator shall not be downgraded by the sponsor. If the sponsor disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor shall be provided with the report.

Causality will be defined according to criteria listed in the ICH E2B guidelines.

Relationship	Description
Related	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

Information for the reporting of suspected unexpected serious adverse reactions:

- a valid trial reference number
- the patient identification code
- an identifiable reporter
- details on the suspected unexpected adverse reaction
- a suspect investigational drug product
- a causality assessment
- the date of the initial information from the primary source
- the date of the receipt of most recent information
- the unique case identification number
- the sender identifier.

Infections:

Infections will be classified, as far as possible, by pathogenic agent, clinical syndrome, localisation and

severity. Date of onset, duration, treatment and outcome will also be recorded.

Cause of death:

Investigators should define death as being caused by SARS-CoV-2 or related to other causes. The cause of death, when identified, should always be reported.

Medical monitoring:

It is the responsibility of the local institutional Principal Investigator to oversee the safety of the trial at the site. The safety monitoring will include assessment and reporting of AE as previously noted, as well as the implementation of a site data and safety monitoring plan. A regular assessment of the number and type of SAE will be part of the medical monitoring.

Contact for pharmacovigilance

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Study Duration: 28 days

Number of Centres: IRCCS Policlinico San Matteo Foundation of Pavia, Italy, and Mario Negri Institute of Bergamo, Italy, are the Co-sponsors of this Investigator Initiated Study.

Co-sponsored Study:

IRCCS Policlinico San Matteo Foundation of Pavia and Mario Negri Institute- BG

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The data safety monitoring committee has no competing interests.

Table 1. Schedule of Assessments.

The following variables will be collected, including demographic data, previous medical history (including the presence and type of comorbidities, smoking history, ongoing treatments), clinical presentation (date and type of symptoms onset leading to the admission to hospital), laboratory parameters (biochemistry, full blood count, IL6 levels, ferritin levels, D-Dimer). Microbiological analyses will be performed to exclude concomitant bacterial infections. Arterial blood gas test (ABG) will be performed. The ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2) will be calculated. Chest radiography (x-ray) will be performed. Serum samples for storage and future assessment of cytokines, cyclophilin, and other exploratory biomarkers.

Study Period:	Screening	Baseline	Treatment period	Follow-up period	End of study
Day	Over first 48 hours after admission of patient	Day 0	Days 0-7	Day 8 – day 15	28 days
Informed Consent	X				
Inclusion/Exclusion Criteria	X	X			
Demographics	X				
Medical History	X				
Physical examination	X	X	X	X	X
Vital signs	X	X	X	X	X
Hematology	X	X	X	X	X
Biochemistry (including CRP,LDH,PCTI)	X	X	X	X	X
Hepatitis B, C tests, HIV	X				
Imaging tests (CXR)	X		X	X	
ABG	X	X	X (Days 1-2-3-4-6-7)	X Day 10	
Adverse events			X	X	X
Blood sample collection for storage (additional cytokine analysis, cyclofillin, CSA trough level and exploratory biomarkers analysis in future)	X		X		X

Procedures at each visit:

Screening visit:

Patients will be screened for eligibility to the study after signing an approved informed consent form. As part of the screening, patient's previous medical history will be recorded. Inclusion/exclusion criteria will be assessed. Vital signs will be recorded (blood pressure, pulse, temperature, respiratory rate, body weight and height). Physical examination and routine laboratory tests will be performed.

Prior and ongoing medications will be recorded. All patients will undergo screening test for the initiation of L-CSA: exclusion of active tuberculosis, HBV serology, HCV serology and if positive, HCV-RNA, HIV. Imaging studies will include chest X ray (CXR). ABG will be performed.

Baseline visit:

Baseline visit will be performed within 48 hours from screening visit. During the proof of concept phase of the study, all eligible patients will receive L-CSA in addition to the standard of care treatment.

Treatment period:

Days 0-7. Patients will receive the active treatment while their inpatient stay. Vital signs will be recorded every 12 hours. Physical examination will be performed every day, while laboratory tests, ABG will be performed at day 2-4-6 and 7and on need, in case of symptoms deterioration.

Follow-up period:

Days 8-15. Patients will be continuously monitored daily until the resolution of COVID-19 infection, or the occurrence of death.

End of study. Patients will be followed up to day 28.

Study Management:

Quality Assurance:

The sponsor is responsible for implementing and maintaining quality assurance with working instructions and adherence to the study protocol.

Case report forms:

Data collection will be performed by the treating physician. Data will be recorded on paper case report forms (CRF) and transferred to electronic CRFs. Participants will not be identified in the CRF by name or initials and birth date. Appropriate coded identification will be used.

Source data will be available at site to document the existence of the study participants. Source data must include the original documents relating to the study, the medical treatment and medical history of the participant. Source documents will be the CRFs, information on AE, SAE, concomitant medications.

Archiving:

Upon completion of the study, data will be archived for a minimum of 10 years on the anonymized dataset. Confidentiality of collected information will be ensured throughout the trial duration.

Data Management:

Data will be pseudo-anonymized, and they will be collected in a secured web-based database in REDCap that will be built and maintained by the Clinical Epidemiology & Biometry Unit on a dedicated server of the Scientific Direction. The Unit will also monitor data quality and completeness and will use the REDCap query facility for interaction with the clinical investigators.

Data will be collected on a secured web-based database in REDCap that will be built and maintained by the Clinical Epidemiology & Biometry Unit on a dedicated server of the Scientific Direction.

Access to the database will be granted nominally to the investigator(s), who will access via username and password (to be renewed every 2 months). No identification data will be recorded in the database. It will be the responsibility of the principal investigator to maintain an updated list of the patient identification data enrolled in the study together with their enrolment number. Regular back up of the database information will be performed automatically.

Data will be extracted through REDCap software. The dataset will be stored by the Clinical Epidemiology & Biometry Unit on a dedicated server of the Scientific Direction.

Single data entry will be performed. Predefined lists of values will be provided for categorical variables; the range for plausible values will be defined for continuous variables. Remote monitoring of missingness will be performed and queries will be sent to the investigator.

Storage of biological material and related health data:

Serum samples will be stored in a biobank located at Policlinico S. Matteo, IRCCS Fondazione, Pavia, Italy. Coded samples without recognizable patient information will be stored with the participants consent.

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