EFFICIENCY IN MANAGEMENT OF ORGAN DYSFUNCTION ASSOCIATED WITH INFECTION BY THE NOVEL SARS-CoV-2 VIRUS (COVID-19) THROUGH A PERSONALIZED IMMUNOTHERAPY APPROACH: THE ESCAPE CLINICAL TRIAL

STUDY PROTOCOL

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EudraCT number: 2020-001039-29

Protocol version: 4.0

Protocol date: 1 April 2020

Study Sponsor:

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DISCLOSURE OF PRINCIPAL INVESTIGATOR

Protocol Study Title: EFFICIENCY IN MANAGEMENT OF ORGAN DYSFUNCTION ASSOCIATED WITH INFECTION BY THE NOVEL SARS-CoV-2 VIRUS (COVID-19) THROUGH A PERSONALIZED IMMUNOTHERAPY APPROACH: THE ESCAPE CLINICAL TRIAL

The herein protocol became known to myself by the Study Sponsor. I understand that the protocol remains as yet unpublished; I certify that all disclosed information to myself for this protocol will remain strictly confidential.

The Principal Investigator,

Print Name

Signature Date

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LIST OF ABBREVIATIONS

AE: adverse event

ALT: alanine aminotransferase

AST: aspartate aminotransferase

EDTA: ethylene-diamene-tetracetic acid

HIV: human immunodeficiency virus

HLA-DR: histocompatibility complex DR

IL: interleukin

IV: intravenous

LPS: lipopolysaccharide

MAS: macrophage activation-like syndrome

MFI: mean fluorescence intensity

PBMCs: peripheral blood mononuclear cells

PCT: procalcitonin

pO_2: partial oxygen pressure

RCT: randomized clinical trial

SAE: serious adverse event

SIRS: systemic inflammatory response syndrome

SOFA: sequential organ failure assessment

TB: tuberculosis

TNFα: tumour necrosis factor-alpha

SYNOPSIS

Aim	Humanity is experiencing since November 2019 a new pandemic by the novel SARS Coronavirus-19 (SARS-CoV-2). A limited subgroup of these patients manifests with severe respiratory dysfunction. Available data suggest macrophage activation syndrome (MAS) or immune dysregulation as the main mechanisms of lung impairment. The present study aims to deliver personalized immunotherapy in order to improve the outcome of lung dysfunction associated with SARS-CoV-2. More precisely, patients infected by SARS-CoV-2 associated with MAS and immune dysregulation will be administered treatment with anakinra or tocilizumab respectively.
Design	Prospective, multicenter, open-label controlled trial
Inclusion criteria	• Age equal to or above 18 years • Male or female gender • In case of women, unwillingness to remain pregnant during the study period. • Written informed consent provided by the patient or by one first-degree relative/spouse in case of patients unable to consent • Confirmed infection by SARS-CoV-2 virus using molecular techniques as defined by the World Health Organization[11] • Organ dysfunction defined as the presence of at least one of the following conditions: – Total SOFA score greater than or equal to 2; – Involvement of the lower respiratory tract • Laboratory documentation of MAS or immune dysregulation. MAS is documented by the findings of any serum ferritin greater than 4,420ng/ml. immune dysregulation is documented by the combination of two findings: a) serum ferritin equal to or lower than 4,420ng/ml; and b) less than 5,000 receptors of the membrane molecule of HLA-DR on the cell membrane of blood CD14-monocytes or less than 30 MFI of HLA-DR on the cell membrane of blood CD14-monocytes as counted by flow cytometry.
Intervention	Patients will receive the standard type of treatment decided by the attending physicians. They will also receive: • In case of diagnosis of MAS, IV anakinra 200mg three times daily (every eight

	hours) for 7 days. Patients who will receive anakinra treatment and who suffer from kidney dysfunction will receive 50% of the dose i.e. 100mg anakinra three times daily for 15 days. • In case of diagnosis of immune dysregulation IV tocilizumab 8mg/kg body weight once up to a maximum of 800mg. These patients will receive anakinra at the above dose in case they meet one of the following contra-indications for tocilizumab: a) absolute neutrophil count less than 2,500/mm^3; b) absolute platelet count less than 100,000/mm^3; and c) AST or ALT more than 1.5 x the upper normal limit
Primary study endpoint	Patients meeting the following composite endpoint which contains the achievement of at least one of the following goals or both goals after 7 days (study visit of day 8): • At least 25% decrease of baseline total SOFA score or increase of the pO_2/FiO_2 ratio by at least 50% • Clinical improvement of lung involvement
Secondary study endpoints	• Comparison of the primary endpoint with historical comparators • Change of SOFA score on day 28 • Mortality on day 28 • Mortality on day 90 • Change of cytokine stimulation between days 0 and 4 • Change of gene expression between days 0 and 4 • Change of serum/plasma proteins between days 0 and 4 • Classification of immune function of screened patients who are not enrolled in study drug since they do not have MAS or immune dysregulation The above secondary endpoints will also be analyzed separately to study the specific effect of anakinra and of tocilizumab.
Number of patients	This is an exploratory trial. Forty (40) patients will be enrolled in total.
Study duration	2 years

BACKGROUND

Humanity is experiencing since November 2019 a new pandemic by the novel SARS Coronavirus-19 (SARS-CoV-2). As of March 16 2020 170,191 documented case were reported worldwide of which 6,526 died[1]. The analysis of the clinical characteristics of these patients showed that among those who were critically ill with acute respiratory failure the risk of death was as high as 60%[2]. Main clinical feature is the presence of comorbidities and age more than 60 years whereas main laboratory findings are leukopenia and lymphopenia with hepatic dysfunction and increase of D-dimers[3,4]. It is also reported that these patients suffer from intense pro-inflammation where hyper-cytokinemia predominates[5,6].

The above characteristics lead to consider two main mechanisms of pathogenesis of this critical condition: macrophage activation syndrome (MAS) and immune dysregulation. Early and correct understanding of the mechanism and management are of prime importance. This can be achieved only through a therapeutic protocol where the early recognition of the immune state can be done with the use of biomarkers and with the delivery of the precise treatment aiming to the correction of the immune dysregulation.

Data of the Hellenic Sepsis Study Group indicate that MAS can be diagnosed with reliability using serum ferritin[7]. Concentrations greater than 4,420ng/ml possess diagnostic specificity 97.3% and negative predictive value 98%. According to these data, the risk of developing MAS is greater among patients with comorbidities like type 2 diabetes mellitus and heart failure who are prone to hyper-production of interleukin (IL)-1β by tissue macrophages[8]. A recent retrospective analysis of patients with severe sepsis and MAS showed that the administration of anakinra decreased 28-day mortality by 30%[9]. Anakinra is the recombinant antagonist of human IL-1β receptor. IL-1β over-production is the hallmark of the pathogenesis of MAS. Results of a phase III study in 906 patients showed that anakinra was a very safe drug: there was neither excess mortality nor increased susceptibility to secondary infections[9]. Since November 2017 the randomized clinical trial entitled «A trial of validation and restoration of immune dysfunction in severe infections and sepsis, PROVIDE» (EudraCT number: 2017-002171-26, approval 78/17 by the National Ethics Committee, approval IS 75/17 by the National Organization for Medicines, ClinicalTrials.gov NCT03332225). In this study patients with sepsis and

laboratory diagnosis of MAS are randomized to treatment with placebo or anakinra for seven days. Enrolment was completed in December 2019 and no drug related adverse events have been reported.

Recent unpublished data of the Hellenic Sepsis Study Group demonstrate that patients with immune dysregulation have profound lymphopenia associated with elevated IL-6. This is in accordance with evidence of the H1N1 pandemic where patients with pneumonia had substantial lymphopenia and increased T regulatory lymphocytes (T_{reg}). This increase of T_{reg} was prominent among patients with comorbidities like diabetes mellitus, chronic heart failure and chronic obstructive pulmonary disease[10,11]. The IL-6 blocker tocilizumab is a promising candidate for the reversal of this immune dysregulation.

ESCAPE is an address to the personalized management of life-threatening organ dysfunction by SARS-CoV-2. More precisely, patients infected by SARS-CoV-2 associated with MAS and immune dysregulation will be administered treatment with anakinra and tocilizumab respectively.

AIM OF THE STUDY

Our aim is to conduct one trial of personalized immunotherapy in patients with SARS-CoV-2 associated with organ dysfunction and with laboratory findings of MAS or immune dysregulation. These patients will be selected using a panel of biomarkers and laboratory findings and they will be allocated to treatment according to their needs.

STUDY DESIGN

This will be a prospective open-label non-randomized study that will take place for 24 months in study sites in Greece (Appendix I). The study protocol will be approved by the National Ethics Committee of Greece and the National Organization for Medicines of Greece. The study will be registered at Clinicaltrials.gov before the enrolment of the first patient.

Study population

Patients who meet ALL the following inclusion criteria and who do not meet any of the following exclusion criteria are allowed to be enrolled:

Inclusion criteria

- Age equal to or above 18 years
- Male or female gender
- In case of women, unwillingness to remain pregnant during the study period.
- Written informed consent provided by the patient or by one first-degree relative/spouse in case of patients unable to consent
- Confirmed infection by SARS-CoV-2 virus using molecular techniques as defined by the World Health Organization[11]
- Organ dysfunction defined as the presence of at least one of the following conditions:
  - Total SOFA score (Appendix II) greater than or equal to 2;
  - Involvement of the lower respiratory tract (Appendix II)
- Laboratory documentation of MAS or immune dysregulation. MAS is documented by the findings of any serum ferritin greater than 4,420ng/ml. Immune dysregulation is documented by the combination of two findings: a) serum ferritin equal to or lower than 4,420ng/ml; and b) less than 5,000 receptors of the membrane molecule of HLA-DR on the cell membrane of blood CD14-monocytes or less than 30 MFI of HLA-DR on the cell membrane of blood CD14-monocytes as counted by flow cytometry.

Exclusion criteria

- Age below 18 years
- Denial for written informed consent
- Any stage IV malignancy
- Any do not resuscitate decision
- Active tuberculosis (TB) as defined by the co-administration of drugs for the treatment of TB
- Infection by the human immunodeficiency virus (HIV)
- Any primary immunodeficiency

- Oral or IV intake of corticosteroids at a daily dose equal or greater than 0.4 mg prednisone or greater the last 15 days.
- Any anti-cytokine biological treatment the last one month
- Medical history of systemic lupus erythematosus
- Medical history of multiple sclerosis or any other demyelinating disorder.
- Pregnancy or lactation. Women of child-bearing potential will be screened by a urine pregnancy test before inclusion in the study

Screening for eligibility

No study related procedure will be performed prior obtaining written inform consent form. Screening can be repeated only if a patient experiences new onset of dyspnea provided that this is taking place within the first 14 days from the diagnosis of infection SARS-CoV-2. Screening is following screening steps:

- Step 1: The patient is screened for the exclusion criteria. If he meets any of them, he cannot be enrolled. If he does not meet any of them, he remains eligible and screening proceeds to step 2
- Step 2: The patient is screened for organ dysfunction. If he does not meet the protocol definition of organ dysfunction, he cannot be enrolled. If he meets the protocol definition of organ dysfunction, he remains eligible and screening proceeds to step 3.
- Step 3: 16ml of whole blood is drawn after venipuncture of one forearm vein under aseptic conditions. The blood is poured into three vials; 2.5 ml into one PAXgene tube; 3ml into one pyrogen-and anticoagulant free tube and another 11.5ml into one EDTA-coated tube containing conservative for flow cytometry. The first tube is centrifuged at 800g at room temperature and the supernatant serum is collected and stored at 4-8°C. Serum samples and tubes with whole blood are transported via courier on the same day to the central lab which is the Research Laboratory of Immunology of Infections of the 4th Department of Internal Medicine at ATTIKON University General Hospital. Upon arrival at the lab, ferritin is measured in the supernatant by an enzyme immunosorbent assay within six hours. In parallel, using EDTA-whole blood, white blood cells are incubated for 15 minutes in the dark with the following monoclonal antibodies: anti-CD14 FITC,

anti-CD45 PC5 and Quantibrite HLA-DR/anti-monocyte PerCP-Cy5.5. Cells are then analyzed through an FC500 flow cytometer against cells stained with anti-CD45 PC5 and anti-idiotypic IgG1. A patient can then be included in the study under two occasions: a) if ferritin is above 4,420 ng/ml which is indicative of MAS; and b) if ferritin is lower than or equal to 4,420 ng/ml and the number of HLA-DR receptors on CD14/CD45-positive cells is lower than 5,000/cells or the mean fluorescence intensity of HLA-DR on CD14/CD45-positive cells is lower than 30 which is indicative of immune dysregulation. Remaining EDTA-blood will be used for the isolation of PBMCs and cytokine stimulation and collection of plasma whilst remaining serum and PAXgene tubes are kept at -80°C until analysis.

Patients who are screened until step 3 and are not included in the study may be screened again for inclusion within the time frame of 14 days defined above.

Allocation to treatment

Patients will receive the standard type of treatment in accordance with the official directive of the National Public Health Organisation[14]. They will also receive:

- In case of diagnosis of MAS, IV anakinra 200mg three times daily at a final volume of 20ml (every eight hours) for 7 days. For patients who suffer from kidney dysfunction: If creatinine clearance is below 30 ml/min they will receive 50% of the dose i.e. 100mg anakinra three times daily for 15 days.
- In case of diagnosis of immune dysregulation IV tocilizumab 8mg/kg body weight once up to a maximum of 800mg. These patients will receive anakinra at the above dose in case they meet one of the following contra-indications for tocilizumab: a) absolute neutrophil count lees than 2,500/mm^3; b) absolute platelet count less than 100,000/mm^3; and c) AST or ALT more than 1.5 x the upper normal limit IV anakinra 200mg three times daily (every eight hours).

Study drug

The active study drug i.e. anakinra and tocilizumab will be provided in the form of pre-filled ready-to-use syringes. All syringes need to be stored at 2-8°C at the study site at a refrigerator with recording of temperature. In case recording indicates deviation of temperature below 0°C or above 10°C for more than a day, stored

syringes need to be replaced by the Sponsor. At the exterior of each syringe there will be a letter and a 6-digit number. The letter refers to the study site, the first two digits of the number refer to the serial number of enrolled patient at the respective study site, the middle two digits of the number refer to the day of treatment and the last two digits of the number refer to the time of treatment. For example, the code A010202 refers to study site A, patient number 01 at that study site, treatment day 2 and second injection for that day.

Patients' visits and interventions (Appendix IV)

Day 1

This visit will take place on the morning of the day of the start of treatment with the study drug. The following procedures will be done on that day:

- Administration of study drug
- Recording of co-morbidities, co-administered drugs, past-history, SOFA score, vital signs, absolute blood cell count (if available) and biochemistry (if available)
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 2

This visit will take place on the morning of the second day from the start of treatment with the study drug. The following procedures will be done on that day:

- Administration of study drug
- Recording of co-administered drugs, SOFA score, vital signs, absolute blood cell count (if available), biochemistry (if available), microbiology and antibiogram (if available)
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 3

This visit will take place on the morning of the third day from the start of treatment with the study drug. The following procedures will be done on that day:

- Administration of study drug
- Recording of co-administered drugs, SOFA score, vital signs, absolute blood cell count (if available), biochemistry (if available), microbiology and antibiogram (if available)

- Recording of adverse events (AE) and severe adverse events (SAE)

Day 4

This visit will take place on the morning of the fourth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Administration of study drug
- Recording of co-administered drugs, SOFA score, vital signs, absolute blood cell count (if available), biochemistry (if available), microbiology and antibiogram (if available)
- 16ml of blood will be collected either from venipuncture of an antecubital vein or directly from a central vein catheter under sterile conditions and distributed as follows: a) 3ml into one sterile and pyrogen-free tube for serum isolation; b) 2.5ml into one PAXgene tube; and c) 11.5ml into one EDTA-coated tube for the isolation of PBMCs and cytokine stimulation and for flow cytometry for CD14(+)/HLA-DR(+)/CD45(+) cells and for plasma collection.
- Recording of any new appearing infection
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 5-7

These visits will take place on the morning of days 5-7 from the start of treatment with the study drug. The following procedures will be done on that day:

- Administration of study drug
- Recording of co-administered drugs, SOFA score, vital signs, absolute blood cell count (if available), biochemistry (if available), microbiology and antibiogram (if available)
- Recording of survival (this can be done by phone call)
- Recording of any new appearing infection
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 8-28

These visits will take place on the morning of days 8-28 from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, SOFA score, vital signs, absolute blood cell count (if available), biochemistry (if available), microbiology and antibiogram (if available)
- Recording of survival (this can be done by phone call)
- Recording of any new appearing infection
- Recording of adverse events (AE) and severe adverse events (SAE)

On day 90 from the start of the study drug, survival is recorded by phone call.

It is clearly stated that a patient may be discharged at the decision of his attending physician any time point after visit 1. In that case, all study procedures described on not performed visits will not be done with the exception of survival recording that can be done by phone call.

LABORATORY PROCEDURES

Isolation of PBMCs

PBMCs will be isolated after gradient centrifugation of whole blood over Ficoll. After serial washing, counting and exclusion of dead cells, they will be stimulated for 24 hours and for five days with LPS, phytohemagglutin and heat-killed *Candida albicans* for the production of TNFα, IL-1β, IL-6, IL-10, IL-17, IL-22 and IFNγ.

Transcriptome, proteome and metabolome analysis

Stored samples from both screened and not enrolled and screened and enrolled patients will be analyzed at the Research Laboratory of Immunology of Infections of the 4th Department of Internal Medicine at ATTIKON University General Hospital or shipped to the Department of Internal Medicine at Radboud University Medical Center, Nijmegen, The Netherlands for analysis. PAXgene tubes will be used for transcriptome analysis, serum/plasma samples for proteome and metabolome analysis.

STUDY ENDPOINTS

Patients meeting the following composite endpoint which contains the achievement of at least one of the following goals or both goals after 7 days (study visit of day 8):

- At least 25% decrease of baseline total SOFA score according to recent suggestion[15] or increase of the pO_2/FiO_2 ratio by at least 50%
- Clinical improvement of lung involvement that is defined as the resolution of all those category 2 and 3 criteria that led to study inclusion (Appendix III)

Patients discharged from hospital alive before study visit of day 8 are considered achieving the primary endpoint. Patients dying before study visit of day 8 are considered non-achieving the primary endpoint.

The secondary study endpoints will be:

- Comparison of the primary endpoint with historical comparators
- Change of SOFA score on day 28
- Mortality on day 28
- Mortality on day 90
- Change of cytokine stimulation between days 0 and 4
- Change of gene expression between days 0 and 4
- Change of serum/plasma proteins between days 0 and 4
- Classification of immune function of screened patients who are not enrolled in study drug since they do not have MAS or immune dysregulation

To facilitate statistical analysis, for patients dying earlier than day 28, SOFA score from the day of death until day 28 will be considered equal to 24 and the pO_2/FiO_2 ratio equal to the one on visit of day 1 before start of the study drug. The above secondary endpoints will also be analyzed separately to study the effect of anakinra and tocilizumab.

NUMBER OF PATIENTS

This is an exploratory trial. Forty (40) patients will be enrolled.

STATISTICAL ANALYSIS

The primary endpoint will be expressed as percentage and 95% confidence intervals. Comparisons with historical cases will be done by the Fisher exact test. Any p-value below 0.05 will be considered significant.

ADVERSE EVENTS

Adverse events (AEs) and Serious Adverse Events (SAEs) will be collected from baseline until the last patient's evaluation. Investigators should monitor subjects for adverse events and are responsible for recording ALL adverse events and serious adverse events occurring to a patient during the trial.

A treatment emergent adverse event (TEAE) is any undesirable medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The time relationship is defined from the moment the TEAE occurs during therapeutic treatment until 30 days or 5 half-lives after treatment discontinuation. The adverse event may be a sign, a symptom, or an abnormal laboratory finding.

Serious adverse events (SAEs) must be reported to within 24 hours. If an adverse event meets any of the following criteria, it is considered SAE:

- **Life-threatening situation** The subject was at risk of death at the time of the adverse event/experience. It does not refer to the hypothetical risk of death if the AE were more severe or were to progress.
- **Inpatient hospitalization** or prolongation of existing hospitalization.
- **Persistent or significant disability/incapacity** Any AE having an outcome that is associated with a substantial disruption of the ability to carry out normal life functions, including the ability to work. This is not intended to include transient interruption of daily activities.
- **Congenital anomaly/birth defects** Any structural abnormality in subject's offspring that occurs after intrauterine exposure to treatment.
- **Important medical events/experiences** that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event/experience when, based upon appropriate medical judgment, **they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above,** i.e., death, a life-threatening

adverse event/experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Examples of such medical events/experiences include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

- **Spontaneous and elective abortions** experienced by study subject.

A non-serious adverse event is any untoward medical occurrence in a patient or subject who is administered a pharmaceutical product, and which does not necessarily have a causal relationship with this treatment. A non-serious adverse event is one that does not meet the definition of a serious adverse event given.

Grading of severity

The severity of the adverse events shall be graded as:

- **Mild** the adverse event is transient and well tolerated by the patient
- **Moderate** the adverse events causes discomfort and affects the usual activities of the patient.
- **Severe** the adverse events affects the usual activities of the patient to an important degree and may cause disability or be life-threatening.

Relationship to the drug

The investigator will use the following definitions to assess the relationship of the adverse event to study drug:

- **Probably Related**: The adverse event has a strong time relationship to the drug or relapses if re-induced, and another etiology is improbable or clearly less probable.
- **Possibly Related**: The adverse event has a strong time relationship to the drug and an alternative aetiology is as probable or less probable.
- **Probably not Related**: The adverse event has a slight or no time relationship to the drug and/or there is a more probable alternative aetiology.
- **Unrelated**: The adverse event is due to an underlying or concomitant disease or to another pharmaceutical product and is not related to the drug (no time relationship and a much more probable alternative aetiology).

If an investigator's opinion of possibly related, probably not related or not related to study drug is given, an alternate etiology must be provided by the investigator. Please note that a severe adverse event/experience is not necessarily serious, as the term severe is a measure of intensity while a serious adverse event is determined based on the aforementioned regulatory criteria. Individual un-blinding thought to be necessary for the management of an adverse event will be documented in the subject Case Report Form.

QUALITY CONTROL AND ASSURANCE

Quality control and assurance checks are performed by sponsor in order to allow periodic review of adequacy of the study activities and practices and allow for revising such practices as needed so the data and process are maintained, the study meets the protocol and procedural requirements, and is reproducible.

Before enrolling any subject in this study, sponsor personnel and the investigator have to review the protocol, the IB, the CRFs and instructions for their completion, the procedure for obtaining informed consent and the procedure for reporting AEs and SAEs.

A qualified representative of the sponsor will monitor the conduct of the study by visiting the site and by contacting the site by telephone and e-mail. During these site visits, all source documents are reviewed, and information recorded in the CRFs is verified against them.

Besides routine monitoring, quality assurance will be documented through independent auditing of the quality control activities and where applicable, by regulatory authorities through inspections.

ETHICAL CONSIDERATIONS

Prior to the initiation of this study, the study design will receive ethical, scientific, and where applicable, regulatory review. Investigators will conduct this study in accordance with the principles of the Declaration of Helsinki, GCP, and applicable regulatory requirements.

Regarding Informed Consent Form obtaining procedures, before any procedure specified in the protocol is performed, a subject must:

- Be informed of all pertinent aspects of the study and all elements of informed consent
- Be given time to ask questions and to consider the decision to participate
- Voluntarily agree to participate in the study
- Sign and date the updated and approved by IEC/REB ICF version.

PROTOCOL ADHERENCE AND AMENDMENTS

Investigators ascertain that they will apply due diligence to avoid protocol deviations. All significant protocol deviations will be recorded and reported in the clinical study report (CSR). Any change or addition to the protocol can only be made in a written protocol amendment that must be approved and signed by the sponsor, health authorities where required, and the IEC/REB.

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APPENDIX I - List of study sites

- 4th Department of Internal Medicine, ATTIKON University General Hospital, Athens (PI: Assoc. Professor Anastasia Antoniadou)
- 2nd Department of Critical Care Medicine, ATTIKON University General Hospital, Athens (PI: Professor Apostolos Armaganidis)
- 1st University Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases, Athens (PI: Professor Antonia Koutsoukou)
- Department of Internal Medicine, Patras University General Hospital, Rion (PI: Professor Charalambos Gogos)
- Intensive Care Unit, General Hospital of Athens IPPOKRATEIO, Athens (PI: Georgios Kofinas, Director of NHS)
- Intensive Care Unit, General Hospital of Athens KORGIALENIO-BENAKIO E.E.S., Athens (PI: Maria Patrani, Director of NHS)
- Intensive Care Unit of LATSEIO Burn Center, THRIASIO Elefsis General Hospital (PI: Nikolaos Markou, Director of NHS)
- Department of Internal Medicine, I PAMMAKARISTOS Hospital, Athens (PI: Ioannis Baraboutis, Director of NHS)
- Intensive Care Unit, KOUTLIMBANEIO & TRIANTAFYLLEIO General Hospital of Larissa, Larissa (PI: Apostolos Komnos, Director of NHS)
- Intensive Care Unit, Ioannina University Hospital, Ioannina (PI: Professor Vasilios Koulouras)
- Intensive Care Unit, General Hospital of Thessaloniki IPPOKRATEIO, Thessaloniki (PI: Eleni Mouloudi, Director of NHS)
- Intensive Care Unit, General Hospital of Thessaloniki O AGIOS DIMITRIOS, Thessaloniki (PI: Glykeria Vlachogianni, Director of NHS)
- Intensive Care Unit, General Hospital of Thessaloniki G. GENNIMATAS, Thessaloniki (PI: Eleni Antoniadou, Director of NHS)
- Intensive Care Unit, THEAGENIO Cancer Hospital of Thessaloniki, Thessaloniki (PI: Souzana Anisoglou, Director of NHS)
- Department of Anesthesiology and Intensive Care Medicine, University General Hospital of Thessaloniki AHEPA, Thessaloniki (PI: Eleni Geka, Director of NHS)
- Department of Internal Medicine, General University Hospital of Larissa, Larissa (PI: Professor George Dalekos)
- Intensive Care Unit, General Hospital ASKLEPIEIO Voulas (PI: Aikaterini Ioakeimidou, Senior Registrar

Monitor Dr. Antigoni Kotsaki, MD, PhD

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APPENDIX II The SOFA score

Variable	0 points	1 point	2 points	3 points	4 points
PaO_2/FiO_2(mmHg)	≥400	<400	<300	<200	<100
Platelets (per mm^3)	≥150	<150	<100	<50	<20
Hypotension	MAP≥ 70 mmHg	MAP<70 mmHg	Dobutamine whatever dose	Adrenaline≤0.1* or Noradrenaline ≤0.1*	Adrenaline>0.1* or Noradrenaline >0.1*
Glasgow Coma Scale	15	13-14	10-12	6-9	<6
Bilirubin (mg/dl)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
Creatinine (mg/dl) or Urine output	<1.2	1.2-1.9	2.0-3.4	35-4.9 or <500ml/day	≥5.0 or <200ml/day

*μg/kg/min

Each variable is scored between 0 and 4. The SOFA score is the sum of the score of each variable

APPENDIX III Diagnostic criteria for lower respiratory tract involvement

Every patient should meet all three categories of criteria in order to be enrolled as having lower respiratory tract involvement

Category 1

Infiltrations compatible with lower respiratory tract infection compatible on chest X-ray or chest computed tomography.

Category 2

Presence of at least 2 of the following criteria

- New onset or worsening of cough
- Dyspnea
- Respiratory rales compatible with lung infection

Category 3

Presence of at least 2 of the following criteria

- PCT ≥0.25 ng/ml
- pO2≤60mmHg or oxygen saturation ≤90% in the air
- Respiratory rate ≥20 breaths/minute

APPENDIX IV Study visits

	Study visits																												
Study day	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**90**
Informed Consent	x																													
Study drug		x	x	x	x	x	x	x																						
SOFA scoring		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Clinical information		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Survival		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Vital signs		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Microbiology		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Blood collection		x			x																								
New infection					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
AE/SAE		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	