IMMUNOTHERAPY NAVIGATED BY SERUM PRESEPSIN FOR INFECTIONS OF THE RESPIRATORY TRACT: THE INSPIRE DOUBLE-BLIND, RANDOMIZED, PHASE IIa EXPLORATORY TRIAL

STUDY PROTOCOL

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DISCLOSURE OF PRINCIPAL INVESTIGATOR

Protocol Study Title: IMMUNOTHERAPY NAVIGATED BY SERUM PRESEPSIN FOR INFECTIONS OF THE RESPIRATORY TRACT: THE INSPIRE DOUBLE-BLIND, RANDOMIZED, PHASE IIa EXPLORATORY 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 I understand that the protocol remains as yet unpublished; I certify that all disclosed information to myself for this protocol will remain strictly confidential.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

AKI: acute kidney injury

ALT: alanine aminotransferase

ARDS: acute respiratory distress syndrome

AST: aspartate aminotransferase

CAP: community-acquired pneumonia

CD14: cluster of differentiation 14

CI: confidence interval

COVID-19: Coronavirus disease 2019

CRA: clinical research associate

CRF: case report form

CSR: case study report

CT: computed tomography

DAMPs: danger-associated molecular patterns

DIC: disseminated intravascular coagulation

ED: emergency department

FiO_2: fraction of inspired oxygen

GCP: good clinical practice

GCS: Glasgow coma scale

GDPR: General Data Protection Regulation

HAP: hospital-acquired pneumonia

HBD: hepatobiliary dysfunction

HISS: Hellenic Institute for the Study of Sepsis

HMGB1: high-mobility group box-1

IB: investigator's brochure

ICAM-1: intercellular adhesion molecule-1

ICF: informed consent form

ICSR: individual case safety report

IL-1: interleukin-1

IUD: intrauterine device

IUS: intrauterine hormone-releasing system

IV: intravenous

LPS: lipopolysaccharide

MAP: mean arterial pressure

mtDNA: mitochondrial deoxyribonucleic acid

NEC: National Ethics Committee

NHS: National Health System

NOM: National Organization for Medicines

PaO_2: partial pressure of arterial oxygen

PBMCs: peripheral blood mononuclear cells

PCT: procalcitonin

PI: principal investigator

qSOFA: quick sequential organ failure assessment

RCT: randomized clinical trial

SAE: serious adverse event

sCD14-ST: soluble cluster of differentiation 14 subset

SoC: standard-of-care

SOFA: sequential organ failure assessment

STEAE: serious treatment-emergent adverse event

SuPAR: soluble urokinase plasminogen activator receptor

SUSAR: suspected, unexpected serious adverse reaction

TEAE: treatment-emergent adverse event

TLR: toll-like receptor

VCAM-1: vascular cell adhesion molecule-1

TRIAL SYNOPSIS

Background and rationale	Data from recent studies of the Hellenic Sepsis Study Group have highlighted that presepsin of more than 350 pg/ml can be a tool for timely diagnosis of severity and prediction of unfavorable outcomes in sepsis. The aim of this study is to evaluate the use of presepsin for guidance of early treatment with anakinra in patients with severe bacterial pneumonia and assess the impact on the clinical outcome.
Study design	Prospective, multicenter, double-blind, randomized, placebo-controlled clinical trial.
Inclusion criteria	• Age equal to or above 18 years • Male or female gender • In case of women of reproductive age, willingness to use dual contraceptive method during the study period • Written informed consent provided by the patient. For subjects without decision-making capacity, informed consent must be obtained from a legally designated representative following the national legislation in the Member State where the trial is planned • Community-acquired pneumonia or hospital-acquired pneumonia • qSOFA score equal to 1 • Serum presepsin > 350 pg/ml
Exclusion criteria	• Age below 18 years • Denial of written informed consent • Any stage IV malignancy • Any do not resuscitate decision • Patients with PaO_2/FiO_2 less than 150 necessitating non-invasive ventilation or mechanical ventilation • Hospitalization in Intensive Care Unit • Known hypersensitivity to anakinra • Oral or IV intake of corticosteroids at a daily dose equal to or greater than 0.4 mg/kg prednisone for a period greater than the last 15 days

	• qSOFA score 0, 2 or 3 • Any anti-cytokine biological treatment for the last one month • Pregnancy or lactation. Women of child-bearing potential will be screened by a urine pregnancy test before inclusion in the study • Participation in any other interventional trial
Intervention	• Treatment Arm 1: patients receiving placebo (N/S 0.9% w/v) subcutaneously once daily for 10 days + SoC • Treatment Arm 2: patients receiving anakinra subcutaneously 100 mg once daily for 10 days + SoC SoC (standard-of-care): It is suggested that all subjects receive combination therapy with a β-lactam/β-lactamase inhibitor or a 3rd generation cephalosporin and a macrolide or monotherapy with a fluoroquinolone. However, other treatments are allowed at the discretion of the attending physicians.
Primary endpoint	The progression into organ dysfunction. This is a composite endpoint. Patients who meet any of the following are considered to meet this endpoint: i) increase of SOFA score by 2 or more points from day 1 (before start of the study drug) until day 7; ii) death by day 90.
Secondary endpoints	• Change of SOFA score over all days of follow-up • Incidence of specific organ dysfunction by day 28 • Time until discharge from hospital • Mortality by day 28 • Mortality by day 90 • Time until attenuation of sepsis-induced inflammation as defined by PCT measurements • Change of presepsin from baseline until day 10 • Comparison of change of cytokine function and endothelial dysfunction markers from baseline by days 4 and 7 • Comparative progression into organ dysfunction (defined as in the primary endpoint) between patients who failed screening because

	of presepsin 350 pg/ml or less and patients who were enrolled in the study and were allocated to Treatment Arm 1 • Comparative 28-day mortality between patients who failed screening because of presepsin 350 pg/ml or less and patients who were enrolled in the study and were allocated to Treatment Arm 1
Power of the study	The needed number of patients is based on the hypothesis that the primary endpoint will be achieved in 65% of treatment group 1 and in 30% of treatment group 2 with 80% power at the 5% level of significance. Therefore, the total number of patients that should be enrolled is 60 with randomization 1:1 (30 patients in Treatment Arm 1; 30 patients in Treatment Arm 2).
Duration	2 years

INTRODUCTION

Sepsis is a potentially lethal syndrome, which is characterized by the dysregulated response of the host to an infection. Due to its severity, sepsis should always be considered in patients with confirmed or suspected infection as it can rapidly progress to organ failure with poor prognosis. Conversely, patients with new-onset organ failure should be suspected for occult infection[1]. Current epidemiology is suggesting an increase in the incidence of new cases[2]. Sepsis has considerable economic burden on the community as septic patients merit higher-level of healthcare and prolonged hospital stay[3]. Subsequently, prompt recognition and treatment are of essence in order to mitigate the overall toll.

In the past years, numerous efforts have been made to identify a biomarker that portends the presence of sepsis, but none has managed to consistently predict which patients will eventually develop this syndrome[4]. This is largely attributed to still-unknown host and pathogen mechanisms by which the sepsis cascade is initiated. Therefore, further understanding of the pathophysiology is of paramount importance.

The pathogenesis of sepsis is multifaceted and includes immune, cardiovascular, coagulation and metabolic perturbations[5]. Immune dysregulation is a well-established component that leads to tissue injury. Activation of the innate immunity is a crucial step in the sequence of the upcoming events[6]. As such, if we manage to early recognize the activation of one specific immune pathway during the initial stages of sepsis in the human host and promptly commence immunotherapy directed against this specific pathway, we may prevent the cascade of events leading the patient to life-threatening organ dysfunction. This paradigm of timely intervention on the immune system upon early recognition of a specific pathway activation is the SAVE-MORE trial in COVID-19. Preemptive initiation of anakinra treatment guided by the early increase of the biomarker suPAR (soluble urokinase plasminogen activator receptor) well before clinical signs of deterioration develop led to a 64% overall improvement and a 55% relative decrease in mortality[7]. This early personalized treatment was registered in December 2021 by the European Medicines Agency.

One similar cascade of events is happening in sepsis. Bacterial lipopolysaccharide (LPS) of the cell membrane of Gram-negative bacteria and danger-associated molecular patterns (DAMPs) like high-mobility group box-1 (HMGB1) and mitochondrial DNA (mtDNA) are recognized by toll-like receptors (TLRs)[5]. Cluster of

Differentiation 14 (CD14) is the naturally occurring receptor of LPS on the surface of monocytes/macrophages and the regulator of TLR-4 signal transduction[8,9]. In 2004, a novel form of CD14, named soluble CD14 subtype (sCD14-ST) or presepsin was found significantly increased in patients with sepsis[10]. Numerous studies have validated its use as an early indicator of sepsis, but a definite cut-off value has not been established due to the heterogeneity in the study design, selection of patients and clinical context[11-13]. Once LPS binds and activates TLR-4, production of interleukin (IL)-1 ensues. As a consequence, early detection of increased presepsin coupled with anakinra, one short half-life inhibitor of the activity of IL-1α and IL-1β, may be a promising personalized treatment strategy for sepsis[14].

In recent years, studies conducted by the Hellenic Sepsis Study Group have shown that presepsin levels over 350 pg/ml have satisfactory diagnostic and prognostic value for sepsis. In particular, results from the INTELLIGENCE-1 study showed that in patients with at least one of the qSOFA criteria, presepsin more than 350 pg/ml has a sensitivity for diagnosing sepsis and 28-day mortality of 80.2% and 91.5%, respectively. Similar results were reproduced by 2 more independent studies; INTELLIGENCE-2, which also included patients with qSOFA ≥ 1 and SAVE trial, which investigated patients with COVID-19[15].

On the other hand, presepsin's role in determining the appropriateness of treatment remains unclear. In a controlled clinical trial conducted by Hongli Xiao et al, presepsin was used at predefined cut-offs in order to modulate the duration of antimicrobial therapy in septic patients. The primary endpoints were the number of days free of antibiotics in a 28-day period and mortality on days 28 and 90. The results revealed significantly fewer days of antibiotic exposure to the presepsin group (14.54 days vs. 11.01 days; $P < 0.001$)[16].

AIM OF THE STUDY

The current study is an exploratory, phase IIa randomized clinical trial (RCT) aiming to evaluate if early presepsin increase coupled with early initiation of anakinra as an adjunct therapy to the standard-of-care treatment may improve outcomes of community-acquired pneumonia or hospital-acquired pneumonia.

STUDY DESIGN

This will be a prospective, multicenter, double-blind, randomized, placebo-controlled clinical trial that will take place in study sites in Greece (APPENDIX I). The study protocol will be approved by the Institutional Review Boards (when required) and by the National Regulatory Authorities. The study will be registered at Clinicaltrials.gov before enrollment of the first patient. The study will be registered at European Union's EudraCT website (https://eudract.ema.europa.eu). The study should comply with:

- Good Clinical Practice (GCP)
- Regulation EU 536/2014
- General Data Protection Regulation (GDPR) EU 2016/679

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 of reproductive age, willingness to use dual contraceptive method during the study period (see paragraph "Contraceptive Measures")
- Written informed consent provided by the patient. For subjects without decision-making capacity, informed consent must be obtained from a legally designated representative following the national legislation in the Member State where the trial is planned
- Community-acquired pneumonia (CAP) or hospital-acquired pneumonia (HAP)
- qSOFA score equal to 1 (APPENDIX II)
- Serum presepsin > 350 pg/ml

CAP definition: new consolidation on chest imaging (simple X-ray or CT scan) in a patient with compatible clinical picture (eg, fever, dyspnea, cough, and sputum production) without any history of contact for two or more days with hospital environment or with health-care facilities for the last 90 days.

HAP definition: new consolidation on chest imaging (simple X-ray or CT scan) in a patient with compatible clinical picture (eg, fever, dyspnea, cough, and sputum production) presenting 48 or more hours after hospital admission, which has not started at the time of admission.

Exclusion criteria

- Age below 18 years
- Denial of written informed consent
- Any stage IV malignancy
- Any do not resuscitate decision
- Patients with PaO_2/FiO_2 less than 150 necessitating non-invasive ventilation or mechanical ventilation
- Hospitalization in Intensive Care Unit
- Known hypersensitivity to anakinra
- Oral or IV intake of corticosteroids at a daily dose equal to or greater than 0.4 mg/kg prednisone for a period greater than the last 15 days
- qSOFA score 0, 2 or 3 (APPENDIX II)
- Any anti-cytokine biological treatment for the last one month
- Pregnancy or lactation. Women of child-bearing potential will be screened by a urine pregnancy test before inclusion in the study
- Participation in any other interventional trial

Screening for eligibility

All patients who present with symptoms of lower respiratory tract infection in the Emergency Department (ED) or in the general ward are potentially eligible to participate in the study. No study-related procedure will be performed prior to obtaining written informed consent form. Screening is performed under the following 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 the first six inclusion criteria. If all these are met, he remains eligible and screening proceeds to step 3

- Step 3: 4 ml of whole blood are drawn after venipuncture of one forearm vein under aseptic conditions and collected into a sterile, EDTA-coated tube. On the same day, samples will be sent from study sites into the study Central Lab (Laboratory of Immunology of Infections, 4th Department of Internal Medicine, ATTIKON University General Hospital); the shipping cost will be covered by the Sponsor. Presepsin is measured in the Central Lab after centrifugation of the sample and isolation of plasma. If presepsin is more than 350 pg/ml, the patient can be enrolled in the trial. The following information should be captured for patients not enrolled in the trial because of presepsin equal to or lower than 350 pg/ml: progression into organ dysfunction; and mortality by day 90.

Randomization

Patients will be randomly assigned 1:1 to treatment Arm 1 and treatment Arm 2. A separate randomization computer-generated chart will be applied in each study site.

- Treatment Arm 1: patients receiving placebo (N/S 0.9% w/v) subcutaneously once daily for 10 days + SoC
- Treatment Arm 2: patients receiving anakinra subcutaneously 100 mg once daily for 10 days + SoC

The drug should be administered at the same time ± 2 hours every day. In case the patient is discharged alive home before the completion of 10 days of treatment, treatment will stop prematurely. It is explicitly stated that the minimum number of days of treatment is four days.

Study drug

The placebo/active comparator will be prepared by one un-blinded pharmacist at each study site. The study team of each study site should have at least one un-blinded study pharmacist and one substitute. Once one patient becomes eligible for study enrollment, the un-blinded pharmacist will be logged-in through one individual username and password into a safe and fully independent page of the study web-portal.

In case the patient is allocated to Arm 1, the un-blinded pharmacist will fill one syringe with 0.67 ml sodium chloride (NaCl) 0.9%. One separate NaCl 0.9% ampule will be provided per patient, per day of Arm 1. The syringe will be same in appearance as the syringe of Arm 2. Then the un-blinded pharmacist will cover the syringe with one corresponding label (see below) and deliver this to one blinded study investigator/study nurse for subcutaneous injection. At the end of the injection, the empty syringe is stored for accountability. In case the patient is allocated to Arm 2, the un-blinded pharmacist will bring one pre-filled ready-to-use syringe at room temperature. Anakinra pre-filled syringes need to be stored at 2-8°C at the study site in a refrigerator with recording of temperature. In case recording indicates a deviation of temperature below 0°C or above 10°C for more than a day, stored syringes need to be replaced by the Sponsor. Then the un-blinded pharmacist will cover the syringe with one corresponding label (see below) and deliver this to one blinded study investigator/study nurse for subcutaneous injection. At the end of the injection, the empty syringe is stored for accountability.

On each label there will be a code composed of nine characters. The first two characters are the letters IN, from the initials of the study. The third and fourth characters are letters and denote the study site. The fifth to seventh characters denote the number of the patient at the specific study site. The eighth and ninth characters refer to the day of treatment. For example, the code INAB00204 refers to study site AB, patient number 002 at that study site on treatment day 4.

Standard-of-care (SoC) treatment

It is suggested that all patients receive at least one of the following antibiotics either single or in combination with one macrolide:

- Ampicillin/sulbactam
- Amoxycillin/clavulanate
- Piperacillin/tazobactam
- Ceftolozane/tazobactam
- Ceftriaxone
- Cefotaxime
- Moxifloxacin

- Levofloxacin

However, the attending physician may modify the treatment based on risk factors for multidrug-resistance pathogens, microbiology results and local epidemiology.

Contraceptive Measures

Suggested contraceptive measures are:

- Male or female condom with or without spermicide
- Cervical cap, diaphragm, or contraceptive sponge with spermicide
- Vasectomized partner
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion

Suggested dual contraceptive measures are:

- Male condom with or without spermicide in combination with: cervical cap, diaphragm, contraceptive sponge with spermicide, intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion
- Female condom with or without spermicide in combination with a vasectomized partner

Hormonal contraception may be combined (estrogen and progestogen containing) associated with inhibition of ovulation (oral, intravaginal, or transdermal), or progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable).

There are none known interactions between the IMP and other medicinal products. Therefore, hormonal contraception may be appropriate, but it is not suggested. If hormonal contraception method is used, it must be supplemented with a barrier method (preferably male condom).

Vasectomized partner is a highly effective birth control method, provided that he is the sole sexual partner of the woman of childbearing potential that participates in the trial and that he has received medical assessment of the surgical success.

Female and male condom should not be used together.

In any case the most effective contraceptive method is sexual abstinence.

Patients' visits

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:

- Recording of co-morbidities, SOFA (sequential organ failure assessment) score (APPENDIX III), co-administered drugs, past-history, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Sampling of 18 ml of venous blood. This will be analyzed as described at the section Laboratory procedures
- Administration of the study drug (placebo/active comparator)

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:

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 8 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

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:

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)

- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 8 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

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:

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 18 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

Day 5

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

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 8 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

Day 6

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

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 8 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

Day 7

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

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 18 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

Day 8

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

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 4 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

Day 9

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

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 4 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

Day 10

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

- Recording of SOFA score (APPENDIX III), co-administered drugs, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (APPENDIX IV)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Sampling of 4 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

Day 14

This visit will take place on the morning of the 14th day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of SOFA score (APPENDIX III)
- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

Day 28

This visit will take place on the morning of the 28th day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

Day 90

This visit will take place on the morning of the 90th day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of survival status
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs

In case of earlier hospital discharge, this will be a phone visit collecting information on the survival status and of non-serious TEAEs and serious TEAEs.

LABORATORY PROCEDURES

Presepsin measurement

PATHFAST Presepsin is a chemiluminescent enzyme immunoassay for the quantitative measurement of presepsin (sCD14-ST) concentration in whole blood or plasma, which yields results within 15 minutes. It will be performed during the screening process and in all collected blood samples.

Procalcitonin measurement

PCT will be measured in all collected samples. The day when values will be lower than 0.5 ng/ml or less than 80% from baseline will be considered as the day of attenuation of sepsis-induced inflammation.

PBMCs isolation

Peripheral blood mononuclear cells (PBMCs) will be isolated via whole blood centrifugation. After serial washing and disposal of cellular debris, PBMCs will be stimulated for 24 hours with LPS, and for 5 days with heat-killed *Candida albicans* for the evaluation of the production of macrophage-derived cytokines, Th1-cytokines, Th2-cytokines and Th17-cytokines.

Endothelial dysfunction markers

The biomarkers that are going to be measured to evaluate endothelial activation are intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin.

The collected blood is aliquoted as follows:

Days 1, 4, 7

a) 4 ml into one sterile, EDTA-coated tube for plasma isolation from which presepsin will be measured
b) 4 ml into one sterile, pyrogen-free tube for serum isolation from which PCT and endothelial dysfunction markers will be measured
c) 10 ml into one EDTA-coated tube for isolation of PBMCs and cytokine stimulation

Days 2, 3, 5, 6

a) 4 ml into one sterile, EDTA-coated tube for plasma isolation from which presepsin will be measured
b) 4 ml into one sterile, pyrogen-free tube for serum isolation from which PCT and endothelial dysfunction markers will be measured

Days 8 to 10 (where applicable)

a) 4 ml into one sterile, EDTA-coated tube for plasma isolation from which presepsin will be measured

Each blood sample tube will be covered with a corresponding label with a code composed of nine characters. The first two characters are the letters IN, from the initials of the study. The third and fourth characters are letters and denote the study site. The fifth to seventh characters denote the number of the patient at the specific study site. The eighth and ninth characters refer to the day from the study drug initiation. For example, the code INAB00204 refers to study site AB, patient number 002 at that study site on treatment day 4. This process is important in order to protect patient's identity. The samples will be sent from study sites to the study Central Lab (Laboratory of Immunology of Infections, 4th Department of Internal Medicine, ATTIKON University General Hospital) on the same day; the shipping cost will be covered by the Sponsor.

STUDY ENDPOINTS

Primary endpoint

This is the progression into organ dysfunction which is a composite endpoint.

Patients who meet any of the following are considered to meet this endpoint: i)

increase of SOFA score by 2 or more points from day 1 (before start of the study drug) until day 7; ii) death by day 90.

Secondary endpoints

These involve the comparisons between the two groups of treatment except for the last two which involve patients who failed screening due to presepsin and those allocated to treatment Arm 1:

- Change of SOFA score over all days of follow-up
- Incidence of specific organ dysfunction by day 28 (APPENDIX IV)
- Time until discharge from hospital
- Mortality by day 28
- Mortality by day 90
- Day until attenuation of sepsis-induced inflammation as defined by PCT measurements (see section Laboratory procedures)
- Change of presepsin from baseline until day 10
- Comparison of change of cytokine function and endothelial dysfunction markers from baseline by days 4 and 7
- Comparative progression into organ dysfunction by day 10 (defined as in the primary endpoint) between patients who failed screening because of presepsin 350 pg/ml or less and patients who were enrolled in the study and were allocated to Treatment Arm 1
- Comparative 28-day mortality between patients who failed screening because of presepsin 350 pg/ml or less and patients who were enrolled in the study and were allocated to Treatment Arm 1

NUMBER OF PATIENTS

The needed number of patients is based on the hypothesis that the primary endpoint will be achieved in 65% of treatment group 1 and in 30% of treatment group 2 with 80% power at the 5% level of significance. Therefore, the total number of patients that should be enrolled is 60 with randomization 1:1 (30 patients in Treatment Arm 1; 30 patients in Treatment Arm 2).

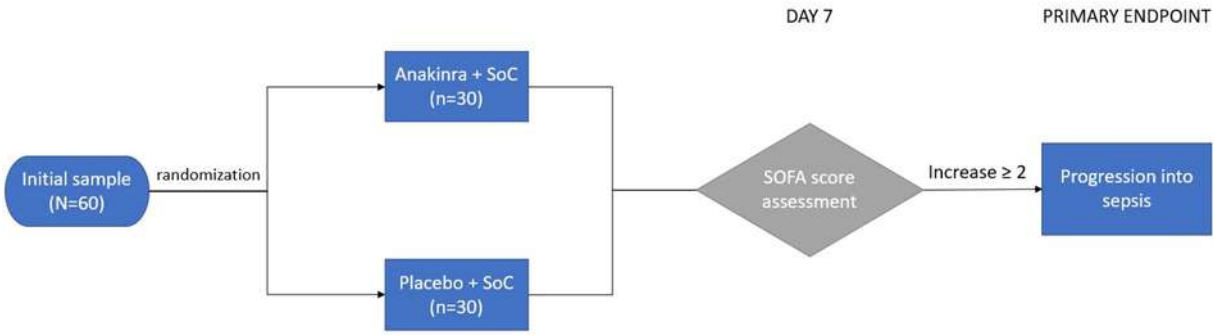

Fig.1 Study flowchart

STATISTICAL ANALYSIS

All baseline quantitative characteristics between the two groups of treatment will be compared by the Student's t-test for parametric variables and by the Mann-Whitney U test for non-parametric variables. Dichotomous variables will be compared by Fischer's exact test. Odds ratio and confidence intervals (CI) will be calculated by Mantel-Haenszel test. Survival will be compared with the log-rank test.

BLINDING PROCEDURES

This study is designed to maintain blinding from participants, site investigators and their teams until completion of the study. At each center, there will be an unblinded pharmacist (and one substitute) who will be in charge of randomizing and preparing the study drug for each participant according to the randomized intervention assignment. These pharmacists will not be involved in data acquisition, collection, adjudication of outcomes or adverse events, or any other study procedures. They will not disclose the treatment assignment to the study team members unless it is via a formal process of early unblinding as described below.

An independent biostatistician will generate the assignment to blinding treatment.

Under normal circumstances, all the treatment assignments of participants will remain blinded until the completion of the trial (completion of enrollment and follow-up or early termination of the trial).

Circumstances for Urgent Unblinding

- **Urgent** unblinding of an individual participant's treatment assignment can be performed for emergency medical reasons to protect participant safety when, as determined by the site Investigator or designee, knowing the participant's treatment assignment would affect immediate medical management of the participant.
- **Non-urgent** early unblinding of an individual participant's treatment assignment for medical/safety reasons, when knowledge of treatment assignment would not affect immediate medical management but may affect other aspects of a participant's medical care/safety.

Reasons and Guidelines for Unblinding

Conventionally, full unblinding of the investigator team takes place after all study data have been recorded and reviewed for all participants, endpoints have been reviewed (if applicable per protocol), and the protocol team has declared the study dataset to be complete and "locked".

It is critical to the objectives of any blinded study that the objectivity of the Investigator team (including site Investigators), other site staff, and participants be maintained. Any unblinding prior to the conventional full unblinding date can result in bias and should therefore be avoided. This includes unblinding the treatment assignments of individual participants as they come off the study, since there is substantial potential for bias in the reporting of results for other participants.

Given that such bias can compromise the integrity or objectivity of the trial, unplanned unblinding prior to the conventional full unblinding date should be undertaken only to protect participant safety or to fulfill safety reporting and other regulatory obligations.

Guidelines for Urgent Unblinding of Individual Participant Treatment Assignments for Medical Reasons

The need for emergency unblinding of individual participant treatment assignments is expected to be extremely rare. Emergency unblinding does not apply for participants who have died, because knowledge of treatment assignment will not affect immediate management in such cases.

Requests for urgent unblinding should be made in writing to the center pharmacist such that he/she can provide the assignment information immediately. In cases of

extreme emergency in which it is not possible for the unblinding request to be made in writing, the site investigator or designee may make the request orally but must provide a written statement of the request within 24 hours, including the reason why the request could not initially be made in writing. In these cases, the center pharmacist can provide the information orally, and provide a written confirmation of the unblinded treatment within 24 hours.

The Site Investigator should alert the Sponsor of the urgent unblinding within 24 hours of the unblinding via email.

Unblinded treatment assignments should be shared with as few individuals as possible on a need-to-know basis. Care should be taken to prevent additional unblinding to maintain study integrity. The site PI and Sponsor are responsible for preventing additional unblinding beyond those who need to know and for protecting information that may identify the participant.

Guidelines for Early Non-Urgent Unblinding of Individual Participant Treatment Assignments for Medical/Safety Reasons

Site PIs or designees may request a participant's treatment assignment before a study is fully unblinded for reasons that are not urgent and do not require immediate (emergency) unblinding but may affect the participant's medical care/safety.

The site PI or designee will consult with the Sponsor via email or teleconference and then submit a written request for unblinding in writing (via email). Early unblinding for this reason should generally not occur until all primary outcome data have been submitted, all queries related to these data have been resolved, and any clinical endpoints have been reviewed by designated reviewers. In cases in which knowledge of a participant's treatment assignment sooner may affect the participant's medical care and/or would otherwise be in the participant's best interest, this requirement can be waived.

ADVERSE EVENTS

Treatment-emergent adverse events (TEAEs) and serious TEAEs (STEAEs) along with AEs/SAEs where applicable will be captured from baseline until the last patient's evaluation. Investigators should monitor subjects for TEAEs and are responsible for recording ALL adverse events and adverse reactions occurring to a

patient during the trial. A TEAE is any undesirable and unintended medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The STEAE may be a sign, a symptom or an abnormal laboratory finding.

If a TEAE meets any of the following criteria, it is considered severe:

- **Death**
- **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 adverse event/adverse reaction were more severe or were to progress
- **Inpatient hospitalization** or prolongation of existing hospitalization
- **Persistent or significant disability/incapacity** Any TEAE 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

Grading of severity

The severity of the TEAE shall be graded as:

- **Mild** the TEAE is transient and well tolerated by the patient
- **Moderate** the TEAE causes discomfort and affects the usual activities of the patient

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

If an investigator's opinion of possibly related, probably not related or not related to the study drug is given, an alternate etiology must be provided by the investigator. Please note that a severe TEAE is not necessarily serious, as the term severe is a measure of intensity. Individual un-blinding thought to be necessary for the management of an adverse event will be documented in the subject Case Report Form (CRF). All Investigators must report every STEA and evaluate the severity and possible causality with the study drug according to the aforementioned criteria. All TEAEs are reported to the Sponsor. The Sponsor is responsible for the evaluation of all TEAEs. All STEAEs/SAEs must be reported within 24 hours of completion of the TEAE and faxed to the Hellenic Institute for the Study of Sepsis (HISS). The Sponsor must evaluate whether a TEAE is expected or not. A STEAE/SAE may qualify for expedited reporting to regulatory authorities if it is determined to be a suspected, unexpected serious adverse reaction (SUSAR). The Sponsor is responsible for submitting expedited safety reports to the appropriate regulatory agency for all confirmed SUSARs. In the case of a fatal or life-threatening SUSAR, the Sponsor will notify the appropriate regulatory agency as soon as possible but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. For a non-life-threatening SUSAR, the report will be submitted no later than 15 days after the Sponsor is made

aware of the event. The Sponsor has the obligation to submit annually a drug safety updated report (DSUR) and an individual case safety report (ICSR) according to global experience to appropriate regulatory authorities. The electronic submission to Eudravigilance will be performed through the Organization ID: HISS.

The above pharmacovigilance procedures will be performed for the Sponsor (Hellenic Institute for the Study of Sepsis) by the Consultant Company SustChem Engineering S.A., 144 3rd Septemvriou str, 11251, Athens, and the Qualified Person for Pharmacovigilance (QPPV) will be Ms Areti Voulomenou, Meng, MSc (contact details: APPENDIX I).

QUALITY CONTROL

The Sponsor will implement quality control and quality assurance control to enable periodic review of the appropriateness of the study activities and practices as required to maintain the quality of data and processes and for the study to abide by the protocol and procedural requirements and be reproducible.

Prior to enrollment of patients in the present study, the Sponsor's staff and the investigator review the protocol, the investigator’s brochure (IB), the CRF and procedures regarding informed consent obtaining and adverse event reporting.

A qualified representative of the Sponsor oversees the conduct of the study with visits to the center and by contacting it via telephone and e-mail. During these visits to the center, all source data are checked and compared with the information recorded in the CRF.

In addition to routine surveillance, quality assurance is documented through independent audits of quality control activities and through inspections by the regulatory authorities, where applicable.

STUDY MONITORING

Clinical research associates (CRAs) of the Sponsor will establish and maintain regular contact between the investigator and the Sponsor. CRAs will evaluate the competence of each study center, informing the Sponsor about any problems relating to facilities, technical equipment or medical staff. During the study, CRAs will check that written informed consent has been obtained from all subjects correctly according to principles of the Declaration of Helsinki, GCP, and applicable regulatory

requirements and that data are recorded correctly and completely. CRAs are also entitled to compare entries in CRFs with corresponding source data and to inform the investigator of any errors or omissions. CRAs will also control adherence to the protocol at the investigator site. They will arrange for the supply of the investigational product and ensure appropriate storage conditions are maintained. The CRAs will make written reports to the Sponsor on each occasion when contact with the investigator is made, regardless of whether it is by e-mail or in person. During monitoring visits, source data verification will be carried out by CRAs and all entries in the CRFs will be compared with the original source documents. The investigator must agree to meet with the CRAs at regular intervals and to cooperate in resolving any queries or findings made during the monitoring process.

ETHICAL ISSUES

Prior to beginning this study, the study design should be subject to ethical, scientific and, where applicable, regulatory review. The researchers will conduct this study in accordance with the principles of the Declaration of Helsinki, the GCP and the current regulatory requirements. In terms of obtaining a written informed consent form (ICF), before any of the procedures identified by the protocol, a patient (or the legally designated representative) must:

- be informed of every aspect of the study and the elements of written ICF
- be given adequate time to ask questions and consider his participation
- have decided voluntarily to participate in the study
- have signed an updated version of the ICF approved by the National Organization for Medicines (NOM)/National Ethics Committee (NEC).

DATA HANDLING AND RECORD KEEPING

Each enrollment center will record patient data in Case Report Forms (CRFs). These forms were designed specifically for the trial according to the study protocol and will be completed for every study participant by a member of the study team.

Source documents will be kept under secure location at individual enrollment centers. Delegated members of the Sponsor will have access to all the records from enrolled participants, for monitoring purposes. All personal data are protected by the

European law Regulation (EU) 2016/679 (General Data Protection Regulation) (GDPR) and Greek law, including law 4624/2019.

All essential data collected will be stored to protect medical confidentiality for a period of twenty-five years according to national requirements after study termination or premature termination of the study.

FINANCING AND INSURANCE

The INSPIRE trial is funded by the Hellenic Institute for the Study of Sepsis (HISS).

PUBLICATION POLICY

The design and primary results of the paper will be published regardless of the study results. HISS will not make final decisions regarding the contents of the publication(s). The results of the trial will also be posted in clinicaltrials.gov and any other public repositories.

PROTOCOL ADHERENCE AND AMENDMENTS

The investigators vouch that they will show due diligence to avoid deviations from the protocol. All significant deviations will be recorded and reported in the clinical study report (CSR). Any changes or additions to the protocol can be made only as a written amendment of the protocol, which must be approved and signed by the Sponsor, the Health Authorities, when required, and the NOM/NEC.

REFERENCES

1. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016; 315: 801-810.
2. Kempker JA, Martin GS. The changing epidemiology and definitions of sepsis. *Clin Chest Med* 2016; 37: 165-179.
3. Paoli CJ, Reynolds MA, Sinha M, Gitlin M, Crouser E. Epidemiology and costs of sepsis in the United States-an analysis based on timing of diagnosis and severity level. *Crit Care Med* 2018; 46: 1889-1897.
4. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. *Crit Care* 2010; 14: R15.
5. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. *Br Med J* 2016; 353: i1585.
6. Angus DC, van der Poll T. Severe sepsis and septic shock. *N Engl J Med*. 2013; 369: 840-851.
7. Kyriazopoulou E, Poulakou G, Milionis H, Metallidis S, Adamis G, Tsiakos K, et al. Early treatment of COVID-19 with anakinra guided by soluble urokinase plasminogen activator receptor: a double-blind, randomized controlled phase 3 trial. *Nat Med* 2021; 27: 1752-1760.
8. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science*. 1990; 249: 1431-1433.
9. Zanoni I, Ostuni R, Marek LR, et al. CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. *Cell*. 2011; 147: 868-880.
10. Yaegashi Y, Shirakawa K, Sato N, et al. Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis. *J Infect Chemother* 2005; 11: 234-238.
11. Masson S, Caironi P, Fanizza C, et al. Circulating presepsin (soluble CD14 subtype) as a marker of host response in patients with severe sepsis or septic shock: data from the multicenter, randomized ALBIOS trial. *Intensive Care Med* 2015; 41: 12-20.
12. Endo S, Suzuki Y, Takahashi G, et al. Usefulness of presepsin in the diagnosis of sepsis in a multicenter prospective study. *J Infect Chemother*. 2012; 18: 891-897.
13. Zhang J, Hu ZD, Song J, Shao J. Diagnostic Value of presepsin for sepsis: a systematic review and meta-analysis. *Medicine* 2015; 94: e2158.

14. Shakoory B, Carcillo JA, Chatham WW, et al. Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of macrophage activation syndrome: reanalysis of a prior phase III trial. *Crit Care Med* 2016; 44: 275-281.
15. Giamarellos-Bourboulis EJ et al. Presepsin as a diagnostic and prognostic biomarker of severe bacterial infections and COVID-19: analysis through one derivation and two validation cohorts; submitted
16. Xiao H, Wang G, Wang Y, et al. Potential value of presepsin guidance in shortening antibiotic therapy in septic patients: a multicenter, prospective cohort trial. *Shock* 2022; 57: 63-71.

APPENDIX I Study sites

- 4th Department of Internal Medicine, ATTIKON University General Hospital, (PI: Antonios Papadopoulos, Professor of Internal Medicine and Infectious Diseases)
- 1st Department of Internal Medicine, General Hospital of Eleusis THRIASIO (PI: Styliani Symbardi, Director of NHS)
- 3rd Department of Internal Medicine, General Hospital of Nikaia AGIOS PANTELEIMON (PI: Ilias Skopelitis, Director of NHS)
- 6th Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases of Athens, (PI: Ioannis Dimitroulis, Director of NHS)
- 1st Department of Internal Medicine, General Hospital of Athens GENNIMATAS (PI: Georgios Adamis, Director of NHS)

Monitor Ms Antigoni Kotsaki
e-mail: a.kotsaki@sepsis.gr, tel: +30 210 74 80 662

Pharmacovigilance supervisor Ms Areti Voulomenou, MEng, MSc
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APPENDIX II qSOFA score

The final score is the sum of points per each clinical parameter.

Clinical parameter	Points
Systolic blood pressure < 100 mmHg	1
Respiratory rate ≥ 22/min	1
Altered mentation GCS <15	1

APPENDIX III 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
Cardiovascular	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	3.5-4.9 or <500ml/day	≥5.0 or <200ml/day

*mcg/kg/min

Each variable is scored between 0 and 4. The SOFA score is the sum of the score of each variable. If there are multiple measurements for each variable per day, calculation is based on the worst value.

APPENDIX IV Definitions of organ dysfunction

Syndrome	**Definition**
Septic shock	All of the following: • Persisting hypotension necessitating vasopressor agents to maintain MAP ≥ 65 mmHg despite adequate volume resuscitation • Lactate > 2mmol/L
DIC	At least two of the following: • International normalized ratio or activated partial thromboplastin time ≥ 1.2 × upper normal value • Platelet count < $100x10^9$/L • Elevated D-dimer
AKI	• Patients without pre-existing renal dysfunction: serum creatinine ≥ 177 mmol/L (≥ 2.0 mg/dL) or Urine volume <0.5 mL/kg/h for 6 hours or need for dialysis • Patients with pre-existing renal dysfunction: ≥ 177 mmol/L (≥ 2.0 mg/dL) increase in serum creatinine or Urine volume <0.5 mL/kg/h for 6 hours or need for dialysis
HBD	At least two of the following: • Total serum bilirubin ≥ 43 mmol/L (≥ 2.5 mg/dL) • Serum concentration of AST or ALT ≥ 2 × upper limit normal • International normalized ratio ≥ 1.5 × upper normal value
ARDS	All of the following: • Bilateral diffuse infiltrates on imaging • PaO_2/FiO_2 < 200 • Need for mechanical ventilation

APPENDIX V Study visits

Visit days	**Screening**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**14**	**28**	**90**
Informed consent	**X**													
Inclusion criteria	**X**													
Exclusion criteria	**X**													
Presepsin measurement	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			
PCT measurement		**X**	**X**	**X**	**X**	**X**	**X**	**X**						
qSOFA	**X**													
SOFA score		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Co-morbidities	**X**													
Co-administered drugs	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			
Culture of PBMCs		**X**			**X**			**X**						
Endothelial dysfunction markers measurement		**X**			**X**			**X**						
TEAE/STEAE		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Organ dysfunction		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			
Survival		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Study drug administration		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			