BIOMARKER-GUIDED EARLY CLARITHROMYCIN TREATMENT TO PREVENT SEPSIS PROGRESSION IN COMMUNITY-ACQUIRED PNEUMONIA: THE REACT RANDOMIZED CLINICAL TRIAL

Evangelos J. Giamarellos-Bourboulis, MD, PhD
Professor of Internal Medicine and Infectious Diseases, National and Kapodistrian University of Athens, Medical School, Greece
President of the Board of the Hellenic Institute for the Study of Sepsis

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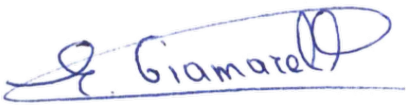

Table of contents

	Page
Trial synopsis	4
Background	9
Aim of the study	12
Study design	13
Study population	13
Screening for eligibility	15
Randomization	16
Standard-of-care treatment	18
Contraceptive measures	19
Patients' visits	20
Laboratory procedures	25
Study endpoints	25
Number of patients	27
Interim analysis	27
Statistical analysis	28
Pharmacovigilance	29
Quality control	31
Study monitoring	32
Ethical issues	33
Data handling and record keeping	33
References	34
Appendix I	36
Appendix II	37
Appendix III	39
Appendix IV	40
Appendix V	43
Appendix VI	44
Appendix VII	45

Abbreviations

CAP: community-acquired pneumonia

COVID-19: coronavirus disease 2019

CSR: clinical study report

DMC: data monitoring committee

ECG: electrocardiogram

ED: emergency department

EMA: European Medicines Agency

EOT: end-of-treatment

ESCMID: European Society for Clinical Microbiology and Infectious Diseases

FDA: Food and Drug Administration

FiO_2: fraction of inspired oxygen

IL: interleukin

LPS: lipopolysaccharide

LRTI: lower respiratory tract infection

PaO_2: partial oxygen pressure

PCT: procalcitonin

PBMCs: peripheral blood mononuclear cells

PSI: pneumonia severity index

RCT: randomized clinical trial

RSS: respiratory symptoms score

SmPC: summary of product characteristics

SoC: standard-of-care

SOFA: sequential organ failure assessment

SSC: surviving sepsis campaign

suPAR: soluble urokinase plasminogen activator receptor

sTEAE: serious treatment-emergent adverse event

TEAE: treatment-emergent adverse event

TNFα: tumour necrosis factor-alpha

TOC: test-of-cure

TRIAL SYNOPSIS

Background and rationale	The primary objective of the REACT RCT is to optimize the clinical benefit from adjunctive clarithromycin treatment shown in the ACCESS trial and to provide evidence for the clinical benefit of early start of adjunctive oral clarithromycin guided by suPAR to prevent the progression into sepsis in patients with CAP at risk. This can be achieved by endpoints incorporating clinical benefit with the effect of treatment on the improvement of the immune dysregulation of CAP. The secondary objectives of REACT are to investigate the impact of early adjunctive treatment with clarithromycin on the resolution of CAP at the test-of-cure (TOC) visit.
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 • Community-acquired pneumonia (CAP) • Presence of at least two of the following signs: i) cough; ii) purulent sputum expectoration; iii) dyspnea; and/or iv) pleuritic chest pain • PCT ≥0.25 ng/ml • suPAR ≥6 ng/ml
Exclusion criteria	• Age below 18 years • Denial of written informed consent • Any stage IV malignancy • Any do not resuscitate decision • Patients necessitating non-invasive ventilation or mechanical ventilation

	• Hospitalization in Intensive Care Unit • Infection by SARS-CoV-2 • 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 • Intake of any macrolide for the current episode of CAP or HAP under study • Known infection by the human immunodeficiency virus • Any chronic anti-cytokine treatment for more than two months • QTc interval at rest in the ECG ≥500 msec or history of know long QT syndrome • QTc interval at rest in the ECG ≥500 msec or history of known long QT syndrome • Medical history of allergy to macrolides • Concomitant oral intake of astemizole, cizapride, doperidone, pimozide, terfenadine, midazolam, ranolazine, ergot alkaloids (e.g. ergotamine and dihydroergotamine), lomitapide and colchicine; patients may be enrolled in the trial if they stop these drugs during trial participation. • Medical history of torsades de pointes arrhythmia • Concomitant intake of lovostatin or simvastatin; patients may be enrolled in the trial if they stop these drugs during trial participation. • Concomitant presence of end-stage liver failure and end-stage renal failure. • Severe hypokalemia or severe hypomagnesemia; a patient may be enrolled one any of these electrolyte disturbances are restored. • Any contradictions for macrolide uptake • 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	Stratified using PSI as a stratum 1: 1 randomization into each of the two treatment arms • Treatment Arm 1: patients receiving oral placebo twice daily for seven days and SoC • Treatment Arm 2: patients receiving oral clarithromycin 500 mg twice daily for seven days and SoC SoC (standard-of-care): It is suggested that all subjects receive therapy with a β-lactam/β-lactamase inhibitor or a 3rd generation cephalosporin or a fluoroquinolone. However, other treatments are allowed at the discretion of the attending physicians.
Primary endpoint	This is defined on day 4 and it is a composite endpoint of three conditions. Only patients meeting ALL three conditions will be considered as succeeding the primary endpoint. ***Condition A*** RSS i.e. the sum of scoring for the symptoms of cough, dyspnea, purulent sputum expectoration and pleuritic chest pain on visit 4 is decreased by at least 50% from the baseline score of day 1 without the development of any new symptom ***Condition B*** SOFA score on visit 4 is decreased by at least 30% from the baseline SOFA score of day 1 ***Condition C*** Combination of: (Plasma PCT on visit 4 has decreased by at least 80% from baseline PCT on screening or it is below 0.25 ng/ml) AND ([plasma IL-10 on visit 4 has decreased by at least 25% from IL-10 of visit 1 or it is below the lower limit of detection] or [the IL-8 to IL-10 ratio of day 4 has decreased less than 15% from the IL-8 to IL-10 ratio of visit 1]). It is explicitly stated that patients dying prior to day 4 are considered failing the primary endpoint.
Key secondary endpoints	• Development of new organ dysfunctions until day 28 • Progression into sepsis
Secondary endpoints	These involve the following comparisons between the two groups of treatment:

	• Clinical success at the TOC visit; this is also analyzed separately for patients infected or colonized by clarithromycin-susceptible and clarithromycin-resistant *S.pneumoniae* • Need for up-escalation of the SoC administered antibiotics. This is considered as the change of the baseline administered SoC antibiotics into more broad-spectrum antibiotics. • Alive hospital discharge until day 28 • Achievement of more than 50% decrease of baseline SOFA score at EOT visit • Score of improvement of CAP-associated immune dysregulation (see sections of Laboratory procedures) at the EOT visit • Change of cytokine production by PBMCs: IL -6, IL-8, IL-10, TNFα on day 4 from visit 1. • Association of 28-day mortality with the score of improvement of CAP-associated immune dysregulation • Association of 90-day mortality with score of improvement of CAP-associated immune dysregulation • Cost of hospital stay
Power of the study	The study is powered for the primary endpoint using the data coming from the ACCESS trial. The power calculation is based on the following assumptions: • All three conditions will be achieved in 20.5% in the placebo and SoC arm • All three conditions will be achieved in 35% in the oral clarithromycin and SoC arm • 80% power • 5% level of significance The final calculation is that 165 patients need to be enrolled in each arm (330 patients in total).
Interim analysis	One interim analysis is planned when 50% of the patient population is enrolled. The analysis will be done by a Data Monitoring Committee (DMC). The following rules will apply in the charter of the DMC:

	Achievement of efficacy. Discontinuation of the study. This will be decided based on the O' Brien and Fleming approach. If the interim analysis has provided achieved a p-value of 0.0054 in favor of clarithromycin for 28-day mortality, the study should be discontinued and the study endpoint should be declared as successful. This leaves a p-value of 0.0492 for the final analysis, which is very close to the desired p-value of 0.05. *Switch to an open-label regime.* In case that the p-value of the interim analysis is larger than 0.0054 but smaller than 0.05 in favor of clarithromycin, the study will switch into open-label and all patients will receive open-label treatment with SoC and oral clarithromycin and comparisons for the primary and secondary endpoint will contain all patients originally allocated to treatment with SoC and placebo and all patients receiving treatment with SoC and oral clarithromycin. *Continuation of the study.* If at the interim analysis the p-value of 0.05 is not achieved, but there will be a difference in 28-mortality of about 5% in favor of clarithromycin yielding a one-side p-value of 0.1 (two-sided p -value ~0.2), the recommendation is for the continuation of the study. *Futility of the study. Discontinuation of the study.* If at the interim analysis, even if clarithromycin yields lower mortality rates, but the one-sided p-value of the Fisher's exact test exceeds 0.15 (two-sided p -value >0.25), or if clarithromycin yields higher mortality rate than placebo, the study is considered futile and the decision should be for discontinuation.
Study duration	2 years (it is anticipated that at least two Winter seasons are necessary)

BACKGROUND

Sepsis is recognized nowadays as one of the major causes of global morbidity and mortality. One analysis of global data revealed that 49 million of new cases are admitted to hospitals every year and that 11 million of them die[1]. Sepsis is the life-threatening organ dysfunction which develops due to the dysregulated response of the host to an infection; mortality is ranging between 20 and 25%[2]. The most common infections leading to sepsis are infections of the lower respiratory tract (LRTIs) mainly community-acquired pneumonia (CAP)[3].

Numerous attempts have been done the last decades to decrease the substantial mortality of sepsis. Standard-of-care (SoC) therapy for sepsis is framed by the Surviving Sepsis Campaign (SSC) guidelines which are published every four years since 2004 (last publication 2021)[4]. The backbone of these guidelines is the early administration of intravenous antibiotics and fluids. In all editions of the SSC guidelines “early” means less than one hour from sepsis recognition. The experts recognize that the secret to decrease mortality is to intervene early before the dysregulated host response to the infection becomes exaggerated and impossible to withhold. They state that for every hour of delay in intervention the odds for survival decrease by relative 7.6%[5]. Then the real ambiguity moves on how the initiation of the sepsis process can be recognized earlier than clinical deterioration appears.

The only way to achieve our current strategy is to implement a strategy linking the increase of a biomarker to an early intervention. This biomarker can be either a measured circulating protein or a bioscore. An example of how this strategy works is the biomarker-guided treatment of early COVID-19 (coronavirus disease 2019) recently approved by the European Medicines Agency (EMA)[6]. The biomarker suPAR (soluble urokinase plasminogen activator receptor) was used as a tool to recognize the patient at risk for severe respiratory failure and guided the start of the drug anakinra. This led to an overall 64% clinical benefit[7] so as to become the first early precision intervention approved for infections by the EMA[8]. The registered cut-off of suPAR which guides early intervention is 6 ng/ml or more.

The suPAR assay is not commercially available in the United States. However, all enrolled patients in SAVE-MORE trial were required to have a plasma soluble urokinase plasminogen activator receptor (suPAR) level ≥ 6 ng/mL. SAVE-MORE was double-blind, randomized controlled trial evaluated the efficacy and

safety of anakinra, an IL-1α/β inhibitor, in 594 patients with COVID-19 at risk of progressing to respiratory failure as identified by plasma suPAR ≥6 ng ml^{-1}, 85.9% (*n* = 510) of whom were receiving dexamethasone. In order to identify a comparable population as was studied in the SAVE-MORE trial, an alternative patient identification method was developed by the Food and Drug Administration (FDA) of the United States to select patients most likely to have suPAR ≥ 6 ng/mL based on commonly measured patient characteristics (Appendix I). This score contains eight clinical variable and patients meeting at least 3 of the variables should receive early anakinra treatment[9,10]. Patients meeting at least three of the following eight criteria are considered likely to have suPAR ≥ 6 ng/mL at baseline: i) age 75 years or more; ii) need for oxygen supplementation; iii) current/previous smoking status; iv) Sequential Organ Failure Assessment (SOFA) score 3 or more; v) neutrophil-to-lymphocyte ratio (NLR) 7 or more; vi) blood hemoglobin 10.5 g/dL or less; vii) medical history of ischemic stroke; and viii) blood urea 50 mg/dL or more and/or medical history of renal disease. According to the SmPC, suPAR is a biomarker is used at the Emergency Department (ED) for the triage of patients for early discharge home or not; levels exceeding 6ng/ml advise for hosptialization[11].

With this precision-embedded approach adjunctive treatments may attenuate the exaggerated response of the host and improve outcomes. Macrolides are a unique class of drugs since they combine both antibiotic and anti-inflammatory properties. Several meta-analyses have proven that addition of a macrolide to treatment decreases mortality by community-acquired pneumonia (CAP)[12]. However, the mortality benefit from macrolides treatment is coming from meta-analyses and no randomized clinical trial (RCT) has been published so far to demonstrate such a benefit. This is also heavily criticized in the recently published guidelines by the European Society of Intensive Care with the European Society of Clinical Microbiology and Infections Diseases (ESCMID) for the management of severe CAP where the recommendation for the co-administration of macrolides to β-lactams is characterized as low-quality evidence[13].

The only available RCT testing the value of early intervention with macrolides in patients with community-acquired pneumonia (CAP) is ACCESS (A randomized clinical trial of oral Clarithromycin in Community-acquired pneumonia to attenuatE inflammatory responseS and improve outcomeS) (EudraCT number 2020-004452-15; ClinicalTrials.gov NCT04724044). The inclusion criteria of the ACCESS trial have

the characteristics of precision therapy i.e. participants were adults with CAP, meeting at least two of the criteria of the systemic inflammatory response syndrome, with SOFA (sequential organ failure assessment) score 2 or more and circulating procalcitonin 0.25 ng/ml or more. ACCESS had a composite primary endpoint pointing towards an early anti-inflammatory clinical benefit of clarithromycin. Patients achieving the endpoint should meet both two conditions A and B by day 4 since start of the study drug: condition A defined as at least 50% decrease of the respiratory symptoms score (RSS); and condition B defined as at least 30% decrease of the baseline SOFA score and/or favorable PCT kinetics (defined as at least 80% decrease of the baseline PCT or PCT less than 0.25 ng/ml). The study succeeded in the primary endpoint since this was met in 38.3% of patients randomized to the SoC + placebo group and in 67.9% of patients randomized to the SoC + clarithromycin group ($p<0.0001$). The ACCESS study also showed remarkable efficacy in all secondary endpoints and mainly to those endpoints pointing towards prevention of sepsis progression:

- 11.30% of patients treated with SoC and placebo progressed into sepsis during the 28-day follow-up compared to only 2.25% of patients treated with oral clarithromycin and placebo (hazard ratio 0.19; p: 0.009)
- 27.8% of patients treated with SoC and placebo developed a new sepsis episode during the 28-day follow-up compared to only 14.8% of patients treated with oral clarithromycin and placebo (hazard ratio 0.48; p: 0.009); and
- 65.4% of patients treated with SoC and placebo were discharged from hospital alive compared to 78.4% of patients treated with oral clarithromycin and placebo (hazard ratio 1.38; p: 0.027)

Post-hoc analysis showed that 87.4% of study participants were meeting at least 3 of the FDA clinical score making them at high likelihood for suPAR 6 ng/ml or more. This means that use of suPAR can discriminate early patients with CAP who are at high likelihood to receive benefit towards prevention of progression into sepsis and organ dysfunction through early start of clarithromycin treatment.

Data from the ACCESS study also showed a significant effect of clarithromycin treatment on modulation of immune dysregulation taking place in CAP by day 4 when the primary endpoint was met. These data points towards improvement of the function of circulating monocytes towards better production of

TNFα (tumour necrosis factor-alpha) after stimulation, decrease of circulating interleukin (IL)-10 and improvement of the IL-8/IL-10 ratio (Figure 1).

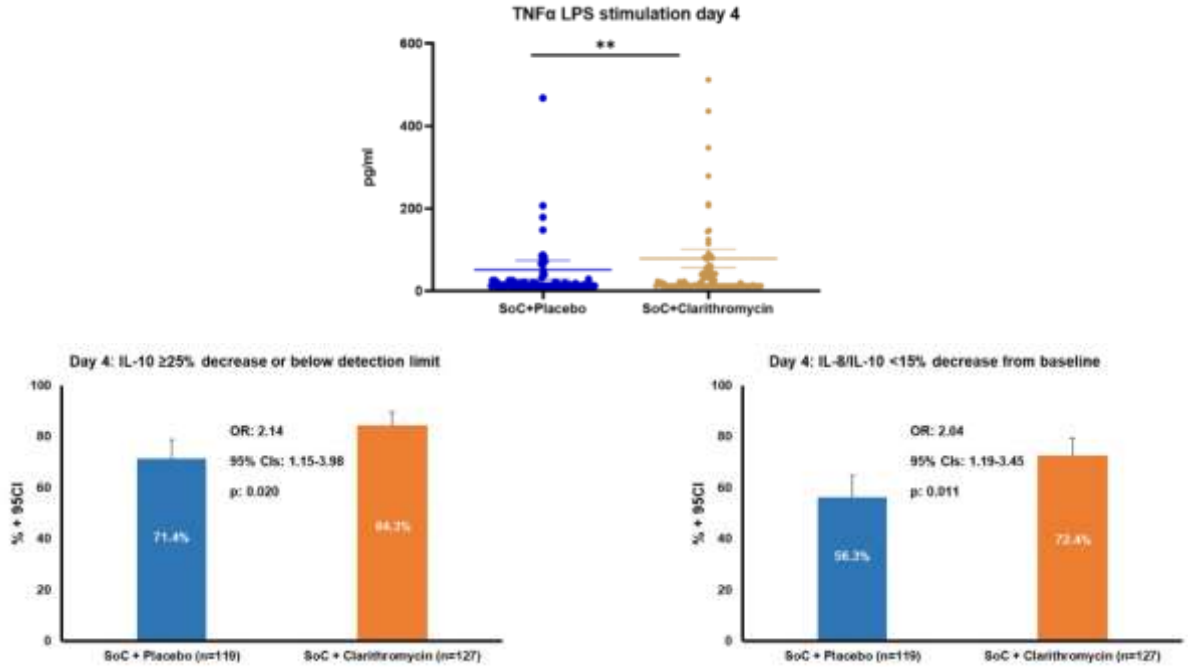

Figure 1 Results of the ACCESS RCT showing the effect of clarithromycin treatment on CAP immune dysregulation by day 4

Upper panel: increase of TNFα production by circulating peripheral blood mononuclear cells after stimulation with bacterial LPS (lipopolysaccharide)

Lower left panel: patients achieving at least 25% decrease of the baseline IL-10 or presenting with serum IL-10 below the limit of detection

Right lower panel: patients achieving less than 15% decrease of the baseline IL-8 to IL-10 ratio

Abbreviations CI: confidence interval; IL: interleukin; n: number of patients; OR: odds ratio; SoC: standard-of-care; TNFα: tumor necrosis factor-alpha

*p of comparison below 0.01

AIM OF THE STUDY

The primary objective of the REACT RCT is to optimize the clinical benefit from adjunctive clarithromycin treatment shown in the ACCESS trial and to provide evidence for the clinical benefit of early start of adjunctive oral clarithromycin guided by suPAR to prevent the progression into sepsis in patients with CAP at risk. This

can be achieved by endpoints incorporating clinical benefit with the effect of treatment on the improvement of the immune dysregulation of CAP. The secondary objectives of REACT are to investigate the impact of early adjunctive treatment with clarithromycin on the resolution of CAP at the test-of-cure (TOC) visit.

STUDY DESIGN

This will be a prospective, multicenter, double-blind, randomized, placebo-controlled clinical trial that will take place in 24 study sites in Greece for two years (Appendix II). The study protocol will be approved by the National Ethics Committee and by the National Organization for Medicines. The study will be submitted using the new CTIS of the EMA. The study will be registered at Clinicaltrials.gov before enrollment 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

1. Age equal to or above 18 years
2. Male or female gender
3. In case of women of reproductive age, willingness to use dual contraceptive method during the study period (see paragraph “Contraceptive Measures”)
4. 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
5. Community-acquired pneumonia (CAP)
6. Presence of at least two of the following signs: i) cough; ii) purulent sputum expectoration; iii) dyspnea; and/or iv) pleuritic chest pain
7. PCT ≥0.25 ng/ml
8. suPAR ≥6 ng/ml

CAP definition: new consolidation on chest imaging 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[14].

Exclusion criteria

- Age below 18 years
- Denial of written informed consent
- Any stage IV malignancy
- Any do not resuscitate decision
- Patients necessitating non-invasive ventilation or mechanical ventilation
- Hospitalization in Intensive Care Unit
- Infection by SARS-CoV-2
- 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
- Intake of any macrolide for the current episode of CAP under study
- Known infection by the human immunodeficiency virus
- Any chronic anti-cytokine treatment for more than two months
- QTc interval at rest in the ECG ≥500 msec or history of known long QT syndrome
- Medical history of allergy to macrolides
- Concomitant oral intake of astemizole, cizapride, doperidone, pimozide, terfenadine, midazolam, ranolazine, ergot alkaloids (e.g. ergotamine and dihydroergotamine), lomitapide and colchicine; patients may be enrolled in the trial if they stop these drugs during trial participation.
- Medical history of torsades de pointes arrhythmia
- Concomitant intake of lovostatin or simvastatin; patients may be enrolled in the trial if they stop these drugs during trial participation.
- Concomitant presence of end-stage liver failure and end-stage renal failure.
- Severe hypokalemia or severe hypomagnesemia; a patient may be enrolled one any of these electrolyte disturbances are restored.
- 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 LRTI in the emergency (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. In case of women of child-bearing potential, urine pregnancy test takes place. These women may proceed to step 2 only if the urine pregnancy test is negative.
- 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: 5 ml of whole blood are drawn after venipuncture of one forearm vein under aseptic conditions and collected into one 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) to be used for the measurement of PCT and suPAR. suPAR is measured by the commercialized quick blood test with suPARnostic® Quick Triage (Virogates S/A, Blokken 45, 3460 Birkerød, Denmark) or suPARnostic® turbilatex, to determinate in 20 minutes levels in human EDTA-plasma. In the Quick Triage test, the sample (10 μl of plasma) will be incubated and handled, according to the manufacturer's instructions, attached to a provided reader and the results will be displayed through LF Software. The measurement is the result of a lateral flow immunoassay (LFIA) and constitutes a quantitative measurement (in ng/ml) of plasma suPAR levels, provided that suPAR values are detected within the range of 2-15 ng/ml, to be considered accurate. If PCT is found ≥ 0.25 ng/ml and suPAR is found ≥6ng/ml, the patient can be enrolled in the study. In case there is no availability for suPAR measurement, the FDA score provided in Appendix I will be used; patients with score 3 or more can be enrolled in the study. The remaining plasma can be stored for future use for proteomic analysis.

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 oral placebo twice daily for seven days and SoC
- Treatment Arm 2: patients receiving oral clarithromycin 500 mg twice daily for seven days and SoC

Randomization will be stratified using one binary variable. The stratum used for randomization will be the pneumonia severity index (PSI) and the strata will be a) PSI above 90; and b) PSI ≤90 (Appendix III).

The study drug should be administered at the same time ± 2 hours every day. In case the patient is discharged alive home before the completion of 7 days of treatment, treatment will continue at home.

The study drug, placebo or clarithromycin, will be provided by the sponsor in the form of tablets in a cartridge. The physician of the center will store and administer the investigational medicinal product in accordance with the instructions provided in the Investigator's Brochure (IB).

In case of hospital discharge, the patient will be provided with the necessary number of tablets in cartridges and an intervention calendar than includes the necessary instructions. At the end of treatment visit, the empty cartridges and calendar will be returned. The administration dosage is dictated by relevant SmPCs. Storage, accountability and destruction will be performed according to the Sponsor's SOPs that will be recorded in given forms. These will be monitored by the Clinical Research Associates and in compliance with GCP standard guidelines.

The side effects associated with clarithromycin treatment are classified as common (≥1/100 and <1/10) and uncommon (<1/100 and ≥1/1000) and they are described below[15]:

System organ class	**Very common (≥1/10)**	**Common (≥1/100 to <1/100)**	**Uncommon (≥1/1,000 to <1/100)**	**Unknown (cannot be estimated from available data)**
Infections and infestations			Cellulitis, candidiasis**,** gastroenteritis, vaginal infection	Pseudomembranous colitis, erysipelas
Blood and lymphatic system			Leukopenia, neutropenia, thrombocytopenia, eosinophilia	Agranulocytosis, thrombocytopenia
Immune system disorders			Anaphylactoid reaction, hypersensitivity	Anaphylactic reaction. angioedema

Metabolism and nutrition disorders			Anorexia, decreased appetite	
Psychiatric disorders		Insomnia	Anxiety, nervousness,	Psychotic disorder, confusional state, depersonalisation, depression, disorientation, hallucination, abnormal dreams, mania
Nervous system disorders		Dysgeusia, headache	Loss of consciousness, dyskinesia, dizziness, somnolence, tremor	Convulsion, ageusia, parosmia, anosmia, paraesthesia
Ear and labyrinth disorders			Vertigo, hearing impaired, tinnitus	Deafness
Cardiac disorders			Cardiac arrest, atrial fibrillation, ECG QT prolonged, extra-systoles, palpitations	Torsades de pointes, ventricular tachycardia, ventricular fibrillation
Vascular disorders		Vasodilation		Hemorrhage
Respiratory, thoracic and mediastinal disorder			Asthma, epistaxis, pulmonary embolism	
Gastrointestinal disorders		Diarrhea, vomiting, dyspepsia, nausea, abdominal pain	Oesophagitis, GRD, gastritis, proctalgia, stomatitis, glossitis, abdominal distension, constipation, dry mouth, eructation, flatulence	Pancreatitis acute, tongue discolouration, tooth discolouration
Hepatobiliary disorders		Liver function test abnormal	Cholestasis, hepatitis, ALT increased, AST increased, γGT increased	Hepatic failure, jaundice hepatocellular
Skin and subcutaneous tissue disorders		Rash, hyperhidrosis	Dermatitis bullous, pruritus, urticaria, rash maculo-papular	Severe cutaneous adverse reactions (SCAR) (e.g. Acute generalised exanthematous pustulosis (AGEP), Stevens-Johnson

				syndrome, toxic epidermal necrolysis, drug rash with eosinophilia and systemic symptoms (DRESS)), acne
Musculoskeletal and Connective tissue disorders			Muscle spasms, musculoskeletal stiffness, myalgia	Rhabdomyolysis, myopathy
Renal and urinary disorders			Blood creatinine increased, blood urea increased	Renal failure, nephritis interstitial
General disorders and administration site conditions	Injection site phlebitis	Injection site pain, inflammation	Malaise, pyrexia, asthenia, chest pain, chills, fatigue	
Investigations			Albumin globulin ratio abnormal, blood alkaline phosphatase increased, blood lactate dehydrogenase increased	International normalised ratio increased, prothrombin time prolonged, urine colour abnormal

Benefit/ risk assessment

The study intervention aims to optimize the clinical benefit from adjunctive clarithromycin treatment shown in the ACCESS trial and to provide evidence for the clinical benefit of early start of adjunctive oral clarithromycin guided by suPAR to prevent the progression into sepsis in patients with CAP at risk. Trial participation can cause, a minor inconvenience from the blood collections. To minimize this, these blood samples will be obtained during routine blood withdrawals where possible. Therefore, it can be safely concluded that, trial participation benefit outweighs any potential risks. In addition benefit/risk assessment will be constantly performed by the Sponsor.

Standard-of-care (SoC) treatment

It is suggested that all patients receive at least one of the following antibiotics based on the current ESCMID guidelines for severe CAP[13]:

- Ampicillin/sulbactam

- Amoxicillin/clavulanate
- Piperacillin/tazobactam
- Ceftriaxone
- Cefotaxime
- Ceftaroline
- Moxifloxacin (only in case urine antigen test is positive for *Legionella* spp or FlimArray is positive for atypical microorganisms)

However, the attending physician may modify the antimicrobial 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). 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

Visit 1: 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 before start of the study drug:

- Recording of co-morbidities, SOFA score (Appendix IV), respiratory symptoms score (RSS, Appendix V) co-administered drugs, past-history, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Calculation of the FDA score for the likelihood of elevated suPAR (Appendix I)
- Collection of urine for antigen detection of *S.pneumoniae* and for *Legionella spp*. This has to be shipped on the same day to the central lab. In case the laboratory identifies positive urine antigen for *Legionella* it has to inform the study site immediately so that SoC treatment is switched into moxifloxacin (see above).
- Collection of sputum and nasopharyngeal swab. Sputum and swab will be plated onto blood agar plates in study sites. Plates and sputum have to be shipped on the same day to the central lab where BioFire Respiratory FilmArray will be done to sputum samples. In case atypical pathogens are isolated in sputum culture and/or in BioFire Respiratory FilmArray, the respective Center must immediately be informed, so that the SoC treatment is switched to moxifloxacin (see above).
- Collection of 20ml of whole blood for indexes of immune function (see chapter laboratory procedures)
- Start of the study drug (placebo/active comparator)

Visit 2: 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 IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)
- Recording of survival status and status of hospitalization
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- 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.

Visit 3: 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 IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)
- Recording of survival status and status of hospitalization
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- 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.

Visit 4: 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 IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if

available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)

- Recording of survival status and status of hospitalization
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Collection of 20ml of whole blood for indexes of immune function (see chapter 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.

Visit 5: 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 IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)
- Recording of survival status and status of hospitalization
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- 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.

Visit 6: 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 IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)
- Recording of survival status and status of hospitalization

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

Visit 7: 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 IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)
- Recording of survival status and status of hospitalization
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- 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.

Visit 8: Day 8: EOT

This is the end of treatment (EOT) visit that is taking place on the next day from end of randomized treatment. The following procedures will be done on that day in rank of order:

- Recording of SOFA score (Appendix IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)
- Recording of survival status and status of hospitalization
- Recording of non-serious treatment-emergent adverse events (TEAE) and serious TEAEs
- Collection of 5ml of whole blood for indexes of immune function (see chapter laboratory procedures)

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.

Visit 9: Day 14: TOC

This is the test-of-cure (TOC) visit that is taking place seven days from end of randomized treatment. The following procedures will be done on that day in rank of order:

- Recording of SOFA score (Appendix IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)
- Any change of the SoC administered antibiotics since visit 8
- Recording of survival status and status of hospitalization
- 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.

Visit 10: Day 28: Survival

This visit that is taking place 21 days from end of randomized treatment. The following procedures will be done on that day in rank of order:

- Recording of SOFA score (Appendix IV), RSS (appendix V) co-administered drugs and antibiotics, complete blood count (if available), biochemistry (if available), microbiology (if available), oxygen saturation, blood gases and new organ failures (Appendix VI)
- Any change of the SoC administered antibiotics since visit 8
- Recording of survival status and status of hospitalization
- 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.

Visit 11: Day 90: Survival

This visit that is taking place 83 ± 2 days from end of randomized treatment.

The following procedures will be done on that day in rank of order:

- Recording of survival status and status of hospitalization
- 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

The collected blood at visits 1 and 4 is poured into two sterile, EDTA-coated tubes of 5ml and 10 ml for the isolation of plasma and peripheral blood mononuclear cells (PBMCs) respectively. The collected blood at visit 8 is poured into two sterile, EDTA-coated tubes of 5ml for the isolation of plasma. Plasma will be used for the measurements of TNFα, IL-6, IL-8, IL-10 and PCT. PBMCs will be stimulated with bacterial LPS for the production of TNFα, IL-6 and IL-10.

On days 4 and 8, the **score of *improvement of CAP-associated immune dysregulation*** is introduced. This the combination of the following changes of circulating inflammatory mediators:

- Plasma PCT has decreased by at least 80% from baseline PCT on screening or it is below 0.25 ng/ml[14]

AND

- [Plasma IL-10 has decreased by at least 25% compared to IL-10 of visit 1 or it is below the lower limit of detection] or [the IL-8 to IL-10 ratio of day 4 has decreased to less than 15% from the IL-8 to IL-10 ratio of visit 1]).

STUDY ENDPOINTS

Primary endpoint

This is defined on day 4 and it is a composite endpoint. This is defined on day 4 and it is a composite endpoint of three conditions (A, B and C). Only patients meeting ALL three conditions will be considered as succeeding the primary endpoint.

Condition A RSS i.e. the sum of scoring for the symptoms of cough, dyspnea, purulent sputum expectoration and pleuritic chest pain on visit 4 is decreased by at

least 50% from the baseline score of day 1 without the development of any new symptom

Condition B SOFA score on visit 4 is decreased by at least 30% from the baseline SOFA score of day 1

Condition C (score of improvement of CAP-associated immune dysregulation) Combination of: (Plasma PCT on visit 4 has decreased by at least 80% from baseline PCT on screening or it is below 0.25 ng/ml) AND ([plasma IL-10 on visit 4 has decreased by at least 25% compared to IL-10 of visit 1 or it is below the lower limit of detection] or [the IL-8 to IL-10 ratio of day 4 has decreased less than 15% from the IL-8 to IL-10 ratio of visit 1]).

The two arms of treatment are compared for this primary endpoint. It is explicitly stated that patients dying prior to day 4 are considered failing the primary endpoint.

Key secondary endpoints

- Development of new organ dysfunctions until day 28
- Progression into sepsis

The two arms of treatment are compared for these key secondary endpoints.

Secondary study endpoints

The comparison between the two arms of treatment in all following endpoints:

- Clinical success at the TOC visit; this is also analyzed separately for patients infected or colonized by clarithromycin-susceptible and clarithromycin-resistant *S.pneumoniae* and it is defined as the complete resolution of RSS
- Need for up-escalation of the SoC administered antibiotics. This is considered as the change of the baseline administered SoC antibiotics into more broad-spectrum antibiotics.
- Alive hospital discharge until day 28
- Achievement of more than 50% decrease of baseline SOFA score at EOT visit
- Score of improvement of CAP-associated immune dysregulation (see sections of Laboratory procedures) at the EOT visit
- Change of cytokine production by PBMCs on day 4 from visit 1

- Association of 28-day mortality with the score of improvement of CAP-associated immune dysregulation
- Association of 90-day mortality with score of improvement of CAP-associated immune dysregulation
- Cost of hospital stay

An overview of study visits is provided in Appendix VII.

NUMBER OF PATIENTS

The study is powered for the primary endpoint using the data coming from the ACCESS trial. The power calculation is based on the following assumptions:

- All three conditions will be achieved in 20.5% in the placebo and SoC arm
- All three conditions will be achieved in 35% in the oral clarithromycin and SoC arm
- 80% power
- 5% level of significance

The final calculation is that 165 patients need to be enrolled in each arm (330 patients in total).

INTERIM ANALYSIS

One interim analysis is planned when 50% of the patient population is enrolled. The analysis will be done by a Data Monitoring Committee (DMC). Analysis will be done for 28-day mortality. The interim analysis will decide upon the continuation of the study as is, the switch to an open-label regime, or the discontinuation on the grounds of achievement of efficacy or futility. The following rules will apply in the charter of the DMC:

Achievement of efficacy. Discontinuation of the study.

This will be decided based on the O' Brien and Fleming approach. Since the achievement of a p-value of 0.05 for the final analysis is of paramount importance for the study the p-value for the interim analysis should be quite small. According to the approach, if the interim analysis will have achieved a p-value of 0.0054 in favor of clarithromycin, the study should be discontinued and the study endpoint should be

declared as successful. This leaves a p-value of 0.0492 for the final analysis, which is very close to the desired p-value of 0.05.

Switch to an open-label regime.

In case that the p-value of the interim analysis is larger than 0.0054 but smaller than 0.05 in favor of clarithromycin, it would be unethical to continue the placebo treatment, since, if the trend is to be continued, the achievement of a p-value of 0.05 is rather guaranteed. In that case, the study will switch into open-label and all patients will receive open-label treatment with SoC and oral clarithromycin and comparisons for the primary and secondary endpoint will contain all patients originally allocated to treatment with SoC and placebo and all patients receiving treatment with SoC and oral clarithromycin.

Continuation of the study.

If at the interim analysis the p-value of 0.05 is not achieved, but there will be a difference in 28-mortality of about 5% in favor of clarithromycin yielding a one-side p-value of 0.1 (two-sided p -value ~0.2), then there is a strong likelihood that the final endpoint will be achieved. Therefore, the recommendation is for the continuation of the study.

Futility of the study. Discontinuation of the study.

If at the interim analysis, even if clarithromycin yields lower mortality rates, but the one-sided p-value of the Fisher's exact test exceeds 0.15 (two-sided p -value >0.25), or if clarithromycin yields higher mortality rate than placebo, the study is considered futile and the decision should be for discontinuation.

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. Missing values for the primary endpoint will be treated as last observation carried forward (LOCF). The primary endpoint will be analyzed by logistic

regression analysis where the stratum used for randomization will be entered as co-variate. Two sensitivity analyses will be done:

- One multivariate logistic regression analysis introducing as co-variates any baseline variables which is statistically different between the two groups will.
- One logistic regression analysis analyzing only the patient population with positive microbiology

Comparison of the key secondary endpoints between the two groups of treatment will be done by Cox-regression analysis. For the analysis of the secondary endpoints of the association of 28-day mortality and 90-day mortality with the score of improvement of CAP-associated immune dysregulation, the odds ratios for the respective mortality in case the score is met will be calculated. The odds ratios will be compared by the Breslow's test and by the Tarone-Ware's test. The cost of hospital stay will be calculated in two ways: using the DRGs (disease related groups) and using the itemized calculations of each service, intervention and administered drugs (as described in references 9, 14 and 16).

PHARMACOVIGILANCE

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 TEAE may be a sign, a symptom or an abnormal laboratory finding.

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

- **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

It is explicitly stated that the elements of the study endpoints i.e. progression into organ failures and death as defined in APPENDIX VI will NOT be reported as TEAEs/sTEEAs.

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 TEA 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. 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.

Once the investigator becomes notified of the emergence of any STEAEs/SAEs, they must report within 24 hours by fax or e-mail or by the e-portal 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 and the Ethics Committee of the study site 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 and the Ethics

Committees of all study sites. 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.

Data protection/ Confidentiality

Data protection and confidentiality will be guaranteed. Data will be handled according to the General Data Protection Regulation (EU) 2016/679 (GDPR). Direct access to source documents will be permitted for purposes of monitoring, audits and inspections. Sponsor eCRFs will be coded to guarantee confidentiality: an identification code number will be used to identify each patient. The subject's identification code will contain 5-digits. This code consists of two letters and a 3-digit number. The letters refer to the study site, the first three digits of the number refer to the serial number of the enrolled patient. Only the Principal investigator (and delegated local study staff) will have access to uncoded data at the site. The Sponsor will only receive coded

information from the study site. Study identification lists will be maintained and updated by the Principal Investigator/delegated personnel.

Public disclosure and publication policy

There is full commitment to defining an appropriate strategy to ensure that the results of the study are published in compliance with the internal provisions for the protection of Intellectual Property Rights (IPR). Prior to the inclusion of the first patient, the study will be registered in a publicly accessible database (ClinicalTrials.gov). To reach the widest possible scientific readership, publication of this study in open-access journals will be ensured.

Within one year after the end of the study, the investigator/sponsor will submit to the competent authorities via CTIS a final report of the study with its results in accordance with CTR Annex IV.

Sample handling

The blood samples collected for PBMCs isolation, serum isolation for PCT measurement and cytokine measurement, will be transported directly to the Central Laboratory at the 4th Department of Internal Medicine at ATTIKON University General Hospital. All collected samples will be labelled with the subject ID code, and the time of sampling. Part of the collected material may be analyzed in other academic centers in Europe.

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. The clinical study falls under regulation EU no 536/2014 (clinical trials regulation).

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/her 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.

REFERENCES

1. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the global burden of disease study. *Lancet* 2020; 395: 200-211.
2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016; 315: 801-810.
3. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, et al. Developing a new definition and assessing new clinical criteria for septic shock: for the third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016; 315: 775-787.
4. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med* 2021; 47: 1181-1247.
5. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; 34: 1589-1596.
6. Karakike E, Giamarellos-Bourboulis EJ, Kyprianou M, Fleischmann-Struzek C, Pletz MW, Netea MG, et al. Coronavirus disease 2019 as a cause of viral sepsis: a systematic review and meta-analysis. *Crit Care Med* 2021; 49: 2042-2057
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. https://www.ema.europa.eu/en/news/ema-recommends-approval-use-kineret-adults-covid-19
9. Akinosoglou K, Kotsaki A, Gounaridi IM, Christaki E, Metallidis S, Adamis G, et al. Efficacy and safety of early soluble urokinase plasminogen receptor plasma-guided anakinra treatment of COVID-19 pneumonia: a subgroup analysis of the SAVE-MORE randomised trial. *eClinicalMedicine* 2023; 56: 101785
10. Fact sheet for healthcare providers: emergency use authorization for kineret https://www.fda.gov/media/163075/download

11. Seppälä S, Andersen AP, Eugene-Olsen J, K. Nyysönen K, Hypöllä H. suPAR cut-offs for stratification of low, medium, and high-risk acute medical patients in the emergency department. *BMC Emerg Med* 2021; 21: 149
12. Nie W, Li B, Xiu Q. β-Lactam/macrolide dual therapy versus β-lactam monotherapy for the treatment of community-acquired pneumonia in adults: a systematic review and meta-analysis. *J Antimicrob Chemother* 2014; 69: 1441-1446
13. Martin-Loeches I, Torres A, Nagavci B, Aliberti S, Antonelli M, Bassetti M, et al. ERS/ESICM/ESCMID/ALAT guidelines for the management of severe community-acquired pneumonia. *Intensive Care Med* 2023; doi: 10.1007/s00134-023-07033-8
14. Kyriazopoulou E, Liaskou-Antoniou L, Adamis G, Panagaki A, Melachroinopoulos N, Drakou E, et al. Procalcitonin to reduce long-term infection-associated adverse events in sepsis. *Am J Resp Crit Care Med* 2021; 15: 202-210
15. SmPC Klaricid https://products.mhra.gov.uk/search/?search=clarithromycin+500+abbott&page=1&doc=Spc&rerouteType=0
16. Tsaganos T, Raftogiannis M, Pratikaki M, Christodoulou S, Kotanidou A, Papadomichelakis E, et al. Clarithromycin leads to long-term survival and cost benefit in ventilator-associated pneumonia and sepsis. *Antimicrob Agents Chemother* 2016; 60: 3640-3646

APPENDIX I. Alternative patient identification method to select patients most likely to have suPAR ≥ 6 ng/ml

Patients meeting at least three of the following eight criteria are considered likely to have suPAR ≥ 6 ng/mL:

1. Age ≥ 75 years
2. Severe pneumonia by WHO criteria
3. Current/previous smoking status
4. Sequential Organ Failure Assessment (SOFA) score ≥ 3
5. Neutrophil-to-lymphocyte ratio (NLR) ≥ 7
6. Hemoglobin ≤ 10.5 g/dL
7. Medical history of ischemic stroke
8. Blood urea ≥ 50 mg/dL and/or medical history of renal disease

APPENDIX II Study sites

- 4th Department of Internal Medicine, ATTIKON University General Hospital (Principal Investigator: Associate Professor Antonios Papadopoulos)
- 2nd Department of Propaedeutic Medicine, ATTIKON University General Hospital (Principal Investigator: Professor Eleni Boutati)
- Department of Chest Medicine, EVANGELISMOS Athens General Hospital (Principal Investigator: Associate Professor Paraskevi Katsaounou)
- 1st Department of Internal Medicine, KORGIALENEION-BENAKEION Athens General Hospital (Principal Investigator: Dr. Vassilki Tzavara)
- 3rd Department of Internal Medicine, KORGIALENEION-BENAKEION Athens General Hospital (Principal Investigator: Dr. Maria Chini)
- 1st Department of Internal Medicine, G. GENNIMATAS Athens General Hospital (Principal Investigator: Dr. George Adamis)
- 3rd University Department of Internal Medicine, SOTIRIA Athens General Hospital of Chest Diseases (Principal Investigator: Associate Professor Garyphallia Poulakou)
- 2nd Department of Pulmonary Medicine, SOTIRIA Athens General Hospital of Chest Diseases (Principal Investigator: Dr. Aggeliki Rapti)
- 6th Department of Pulmonary Medicine, SOTIRIA Athens General Hospital of Chest Diseases (Principal Investigator: Dr. Ioannis Dimitroulis)
- 1st Department of Internal Medicine, THRIASIO Eleusis General Hospital (Principal Investigator: Dr. Styliani Sympardi)
- 2nd Department of Internal Medicine, THRIASIO Eleusis General Hospital (Principal Investigator: Dr. Zoi Alexiou)
- 1st Department of Internal Medicine, EVANGELISMOS Athens General Hospital (Principal Investigator: Dr. Theano Kontopoulou)
- 1st Department of Internal Medicine, SISMANOGLEIO-AMALIA FLEMING Attica General Hospital, Athens (Principal Investigator: Dr. Nikolaos Tsokos)
- 2nd Department of Internal Medicine, TZANEION Piraeus General Hospital (Principal Investigator: Dr. George Chrysos)
- 1st Department of Internal Medicine, LAIKON Athens General Hospital (Principal Investigator: Associate Professor Mina Psychogiou)

- Department of Pathophysiology, LAIKON Athens General Hospital (Principal Investigator: Professor Nikolaos Sypsas)
- 1st University Department of Internal Medicine, SOTIRIA Athens General Hospital of Chest Diseases (Principal Investigator: Professor Petros Bakakos)
- Department of Emergency Medicine, TZANEION Piraeus General Hospital (Principal Investigator: Dr. Styliani Gerakari)
- 1st Department of Internal Medicine, Ioannina University General Hospital (Principal Investigator: Professor Haralampos Milionis)
- Department of Internal Medicine, Larissa University General Hospital (Principal Investigator: Professor George N. Dalekos)
- 2nd Department of Internal Medicine, Alexandroupolis University General Hospital (Principal Investigator: Associate Professor Periklis Panagopoulos)
- Department of Pulmonary Medicine, General Hospital of Kerkyra (Principal Investigator: Dr. Ilias Papanikolaou)
- Department of Internal Medicine, Patras University General Hospital (Principal Investigator: AssociateProfessor Karolina Akinosoglou)
- 1st Department of Internal Medicine, AHEPA Thessaloniki University General Hospital (Principal Investigator: Professor Simeon Metallidis)

Monitor Dr. Antigoni Kotsaki, MD, PhD

e-mail: a.kostaki@sepsis.gr tel: +30 694 6637164; +30 210 58312562

Pharmacovigilance supervisor Ms Areti Voulomenou, MEng, MSc

e-mail: voulomenou@suschem.gr Tel: +30 210 82 52 510, Fax: +30 210 8252575

APPENDIX III Calculation of the pneumonia severity index

Demographics	**Points assigned**
If male	Equal to age
If female	Equal to age -10
Nursing home resident	+10
Comorbidity	
Neoplastic disease	+30
Liver disease	+20
Congestive heart failure	+10
Cerebrovascular disease	+10
Renal disease	+10
Physical exam findings	
Altered mental status	+20
Pulse ≥125 beats/min	+20
Respiratory rate ≥30 min	+20
Systolic blood pressure <90 mmHg	+15
Temperature <35°C or ≥40°C	+10
Lab and radiology findings	
Arterial pH<7.35	+30
Blood urea nitrogen ≥30mg/dl (or 9mmmol/l)	+20
Sodium <130 mmol/l	+20
Glucose ≥250 mg/dl (14mmol/l)	+10
Hematocrit <30%	+10
Partial pressure of oxygen <60mmHg	+10
Pleural effusion	+10
PSI class I	**Total points ≤50**
PSI class II	**Total points 51-70**
PSI class III	**Total points 71-90**
PSI class IV	**Total points 91-130**
PSI class V	**Total points >130**

APPENDIX IV SOFA score calculation

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.

Please note the following:

- For each variable, use the worse measurement available that day before the time of the visit
- In the case one variable value for the calculation of SOFA is not available on one day, please use the immediately previously available value
- In the case, the blood gases values are not available on a specific day of follow-up please use the value of oxygen saturation and FiO_2 as described in the Tables below:

Table Estimating partial oxygen pressure from a given oxygen saturation:

Oxygen saturation (%)	**Corresponding partial oxygen pressure**
80	44
81	45
82	46
83	47
84	49
85	50
86	52
87	53
88	55
89	57
90	60
91	62
92	65
93	69
94	73
95	79
96	86
97	96
98	112
99	145

Table Estimating fraction of inspired oxygen from the mode of oxygenation:

Method	**Oxygen flow (l/min)**	**Fraction of inspired oxygen (%)**
Nasal cannula	1	24
	2	28
	3	32
	4	36
	5	40
	6	44
Nasopharyngeal catheter	4	40
	5	50
	6	60
Face mask	5	40
	6-7	50
	7-8	60
Face mask with reservoir	6	60
	7	70
	8	80
	9	90
	10	95

APPENDIX V The respiratory symptoms score

Symptom	Absent (score=0)	Mild (score=1)	Moderate (score=2)	Severe (score=3)
Cough	No cough or resolution (to pre-CAP levels)	Cough present but it does not interfere with subject's usual daily activities	Cough present, frequent and it does interfere with some of the subject's usual daily activities	Cough is present throughout the day and night; it limits most of the subjects' usual daily activities and sleep patterns
Chest pain	No chest pain or resolution of chest pain related to CAP	Chest pain present occasionally with deep breathing but it does not interfere with subject's usual daily activities	Chest pain is present with normal breaths and it does interfere with the subject's usual daily activities	Chest pain is present at rest and/or with shallow breathing; it limits most of the subject's usual daily activities
Shortness of breath (dyspnea)	No shortness of breath or resolution (to pre-CAP Baseline)	Shortness of breath with strenuous activities only but it does not interfere with subject's usual daily activities	Shortness of breath with usual activities and it does interfere with the subject's usual daily activities	Shortness of breath with minimal exertion or at rest; it limits most of the subject's usual daily activities
Sputum	No coughing up of phlegm/sputum or resolution (to pre-CAP Baseline)	Subject coughs up a small amount of phlegm/sputum	Subject coughs up a moderate amount of phlegm/sputum	Subject coughs up a large amount of phlegm/sputum

APPENDIX VI 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
Disseminated intravascular coagulation	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
Acute kidney injury	• 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
Hepatobilliary dysfunction	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
Aacute respiratory distress syndrome	All of the following: • Bilateral diffuse infiltrates on imaging • PaO_2/FiO_2 < 200 • Need for mechanical ventilation

APPENDIX VII Activities performed during study visits

Visits	**Screening**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**EOT**	**TOC**	**10**	**11**
Days		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**14**	**28**	**90**
Informed consent	x											
Inclusion criteria	x											
Exclusion criteria	x											
PCT measurement	x				x				x			
suPAR measurement	x											
FDA score for suPAR likelihood		x										
Lower respiratory FilmArray		x										
S.pneumoniae urine antigen		x										
Leginella urine antigen		x										
Sputum cultures		x										
SOFA score		x	x	x	x	x	x	x	x	x	x	
Cytokines in plasma		x			x				x			
PBMC stimulation		x			x							
Co-morbidities		x										
Co-administered drugs		x	x	x	x	x	x	x				
TEAE/sTEAE			x	x	x	x	x	x	x	x	x	x
Organ dysfunction			x	x	x	x	x	x	x	x	x	
Survival			x	x	x	x	x	x	x	x	x	x
Study drug administration		x	x	x	x	x	x	x				

APPENDIX VIII Study design flowchart

