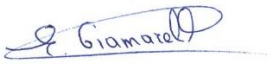
Hellenic Sepsis Institute for the Study of Sepsis (HISS)

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

Statistical Analysis Plan (SAP)

Date prepared	**18 February 2025**
Version number	**1.0**
Prepared by (Lead Statistician)	**Pavlina Mesiri**
Approved by	**Evangelos J. Giamarellos-Bourboulis**
	
Date approved	**28 February 2025**

EudraCT Number: 2023-507295-40-00

ClinicalTrials.gov Number: NCT06294600

Table of Contents

Table of Contents **2**
LIST OF ABBREVIATIONS 3
1. Summary description of the study 4
2. Population and baseline characteristics 6
3. Concomitant therapy 9
4. Study Endpoints 10
4.1 Primary Endpoint 10
4.1.1 Post-hoc analysis of Primary Endpoint 12
4.1.2 Sensitivity Analysis 13
4.2 Key secondary efficacy endpoints 13
4.2.1 Time until progression into organ dysfunction by Day 28 13
4.2.2 Time until development of new sepsis by Day 28 14
4.3 Secondary efficacy endpoints 15
4.3.1 Clinical success at the Test of Cure (TOC) visit 15
4.3.2 Up-Escalation of Antimicrobial Treatment 15
4.3.3 Alive hospital discharge until Day 28 16
4.3.4 Achievement of more than 50% reduction from baseline SOFA score at the End-of-Treatment (EOT) visit 16
4.3.5 Achievement of the predefined score of improvement of CAP-associated immune dysregulation at the EOT visit 17
4.3.5.1 Additional analysis of achievement of the predefined score of improvement of CAP-associated immune dysregulation at the EOT visit 18
4.3.6 Change in cytokine production by peripheral blood mononuclear cells (PBMCs) on Day 4 compared with Visit 1 19
4.3.7 Association of 28-day mortality with the score of improvement of CAP-associated immune dysregulation 20
4.3.8 Association of 90-day mortality with the score of improvement of CAP-associated immune dysregulation 20
4.4 Analyses not provisioned in the protocol 21
5. Toxicity/safety 23

LIST OF ABBREVIATIONS

AE, adverse event

ALT, alanine Aminotransferase

aPTT, activated partial thromboplastin time

AST, aspartate aminotransferase

CAP, community-acquired pneumonia

CCI, charlson comorbidity index

DMC, data monitoring committee

DRGs, disease related groups

eCRF, electronic case-report form

EOT, end-of-treatment

FiO2, fraction of inspired oxygen

γGT, Gamma-glutamyl transferase

IL, interleukin

LOCF, last observation carried forward

LPS, lipopolysaccharide

LRTI, lower respiratory tract infection

NPV, negative predictive value

PaO2, partial oxygen pressure

PCT, procalcitonin

PBMCs, peripheral blood mononuclear cells

PSI, pneumonia severity index

PPV, positive predictive value

RSS, respiratory symptoms score

SAE, serious adverse event

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

1. Summary description of the study

Study objectives	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.
Phase	III
Sample size	330 patients
Intervention arms (what strategy was followed)	
Treatment Arm 1	Oral placebo twice daily for seven days and SoC
Treatment Arm 2	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 analysed 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 antibiotic
	• 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 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

2. Population and baseline characteristics

The following should be presented:

- Flowchart of patients in the trial showing the number of patients from enrolment to analysis including the number of patients who were screened, screened failed and the reasons why they were ineligible (Inclusion/Exclusion criteria)
- All analysis except Safety will be Intention to Treat (ITT). The ITT set will consist of all patients who were randomized and did not withdraw consent nor requested removal of all their data.
- The Safety analysis will be performed on the Safety Analysis Set (SAF) which will consist of all patients who are randomized and received at least one dose of the study drug.
- Baseline Characteristics, baseline respiratory symptoms, medical history, baseline laboratory values, baseline positive microbiology and antibiotics in baseline SoC in each study group:
    - For categorical variables, each column will contain N (%)
    - For continuous variables, the normality assumption of the variables will be examined using the Shapiro-Wilk test. If p-value of the Shapiro-Wilk test is ≥ 5%, the data will be presented as mean (standard deviation [SD]). If the p-value is less than 5%, the data will be presented as median (interquartile range [IQR]).
    - P-values for comparing the study groups will be reported. Student's t-test will be used to compare the 2 treatment groups for the baseline variables which follow the Normal distribution (parametric variables) and by the Mann-Whitney U test for non-Normal variables (non-parametric variables). Dichotomous variables will be compared by Fischer's exact test

  The Table to be presented is drafted using the following template:

Table Baseline Characteristics of Participants in the REACT Trial

	Placebo + SoC (n=xxx)	**Clarithromycin + SoC (n=xxx)**	**P-value**
Male/Female, n (%)	n (%) / n (%)	n (%) / n (%)	x.xxx
Age, years	x.x (x.x)	x.x (x.x)	x.xxx
BMI (kg/m2)	xx.xx (x.xx)	xx.xx (x.xx)	x.xxx
PSI	xxx.x (xx.x)	xxx.x (xx.x)	x.xxx
≤90, n (%)	xxx (xx.x%)	xxx (xx.x%)	x.xxx
>90, n (%)	xxx (xx.x%)	xxx (xx.x%)	x.xxx
CCI	x.xx (x.xx)	x.xx (x.xx)	x.xxx
SOFA score	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
PCT, ng/ml	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
suPAR, ng/ml	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
SCORE2	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Baseline respiratory symptoms			
Cough, n (%)	xxx (xx.x%)	xxx (xx.x%)	x.xxx
Chest pain, n (%)	xxx (xx.x%)	xx x(xx.x%)	x.xxx
Dyspnea, n (%)	xxx (xx.x%)	xxx (xx.x%)	x.xxx
Sputum production, n (%)	xxx (xx.x%)	xxx (xx.x%)	x.xxx
RSS	x.x (x to x)	x.x (x to x)	x.xxx
Time (days) between start of symptoms and study enrolment	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Time (days) between CAP diagnosis and study enrolment	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Medical history, n (%)			

Diabetes mellitus type 1 or 2	xx (xx.x%)	xx (xx.x%)	x.xxx
Chronic obstructive pulmonary disease	xx (xx.x%)	xx (xx.x%)	x.xxx
Chronic heart failure	xx (xx.x%)	xx (xx.x%)	x.xxx
Chronic renal disease	xx (xx.x%)	xx (xx.x%)	x.xxx
Coronary heart disease	xx (xx.x%)	xx (xx.x%)	x.xxx
Atrial fibrillation	xx (xx.x%)	xx (xx.x%)	x.xxx
Smoking	xx (xx.x%)	xx (xx.x%)	x.xxx
Coronary-aorta bypass surgery	xx (xx.x%)	xx (xx.x%)	x.xxx
Stroke	xx (xx.x%)	xx (xx.x%)	x.xxx
Baseline laboratory			
pO_2/FiO_2	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Absolute WBC count, /mm^3	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Platelets, $x10^3/mm^3$	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
C-reactive protein, mg/l	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Neutrophils, %	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Lymphocytes, %	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Hemoglobin, g/dl	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
International normalized ratio	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
aPTT, secs	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Triglycerides, mg/dl	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx

Fibrinogen, mg/dl	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Creatinine, mg/dl	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Total bilirubin, mg/dl	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
AST, U/l	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
ALT, U/l	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
γGT, U/l	mean (SD) or median (Q1 to Q3)	mean (SD) or median (Q1 to Q3)	x.xxx
Total patients with positive microbiology, n (%)	n (xx.x)	n (xx.x)	x.xxx
Antibiotics in baseline SoC, n (%)	xx (xx.x)	xx (xx.x)	x.xxx

Abbreviations: BMI, Body Mass Index; CCI, Charlson's comorbidity index, FiO_2, fraction of inspired oxygen; n, number of patients; PCT, procalcitonin; pO_2, partial oxygen pressure; PSI, pneumonia severity index; Q, quartile; SD, standard deviation; SoC, standard-of-care; SOFA: Sequential Organ Failure Assessment; suPAR, soluble urokinase plasminogen activator receptor; RSS, respiratory symptoms score

- List any possible major protocol deviations or ineligibility criteria that would lead to the patient not being included in the analysis

3. Concomitant therapy

All concomitant medications will be recorded in the medical file by the investigator and on the eCRF platform of the study. Concomitant medications/therapy: medications taken or procedures performed from time of informed consent until 90 days after the last study treatment will be considered concomitant treatment. Concomitant medication will be listed.

4. Study Endpoints

4.1 Primary Endpoint

Condition A of the Primary Endpoint refers to the RSS decrease at Day 4 from the baseline (Day 1) and will be calculated for each patient as follows:

$$RSSdiff = \frac{\text{RSSday4} - \text{RSSday1}}{\text{RSSday1}} * 100$$

The RSS is the sum of scoring for the symptoms of cough, dyspnea, purulent sputum expectoration and pleuritic chest pain. The scoring of each symptom is captured in the eCRFs. If RSS is decreased by at least 50% and the patient hasn't developed any new symptoms then Condition A is considered satisfied.

$RSSdiff$ range can be from -∞ to +∞.

Condition B of the Primary Endpoint refers to the SOFA score decrease at Day 4 from the baseline (Day 1) and is calculated for each patient as follows:

$$SOFAdiff = \frac{\text{SOFAday4} - \text{SOFAday1}}{\text{SOFAday1}} * 100$$

The SOFA score is captured in the eCRFs.

$SOFAdiff$ range can be from -∞ to +∞.

If SOFA is decreased by at least 30% then Condition B is considered satisfied.

Condition C of the Primary Endpoint refers to the score of improvement of CAP-associated immune dysregulation and is computed as follows:

$$PlasmaPCTdiff = \frac{\text{PlasmaPCTday4} - \text{PlasmaPCTscreening}}{\text{PlasmaPCTscreening}} * 100$$

A new categorical variable [pctruleday4 (Yes/No)] will then be created. The variable will be equal to "Yes" if $Plasma\ PCT$ is decreased by at least 80% or Plasma PCT on Day 4 is below 0.25 ng/ml. If none of the conditions are satisfied, then "pctruleday4" is "No".

$$\text{IL10 } diff = \frac{\text{IL10day4} - \text{IL10day1}}{\text{IL10day1}} \text{*100}$$

A new categorical variable [il10day4rule (Yes/No)] will then be created. The variable will be equal to “Yes” if $\text{IL10 } diff$ is decreased by at least 25% or plasma IL10day4 is below the lower limit of detection. If none of the conditions are satisfied, then “il10day4rule” will be “No”.

$$\text{IL8/IL10 ratio } = \frac{\frac{\text{IL8}}{\text{IL10}}\text{day4} - \frac{\text{IL8}}{\text{IL10}}\text{day1}}{\frac{\text{IL8}}{\text{IL10}}\text{day1}} \text{*100}$$

A new categorical variable [il8il10decrease15day4 (Yes/No)] will then be created. The variable will be equal to “Yes” if $\frac{\text{IL8}}{\text{IL10}}\text{ratio } is\ decreased$ by at least 15% from the IL8/IL10 ratio ratio of Day 1 or “No” otherwise.

Condition C is considered satisfied only if the following apply:

[(Variable pctruleday4 = “Yes” and (variable il10day4rule = “Yes” and/or variable il8il10decrease15day4 = “Yes”)]

Only patients meeting ALL three conditions (Condition A & Condition B & Condition C) will be considered as succeeding the primary endpoint.

It is explicitly stated that patients dying prior to Day 4 are considered failing the primary endpoint.

The two arms of treatment will be compared for the primary endpoint first using the Fisher exact test, where any 2-sided p-value less than 0.05 will be considered statistically significant. Then definite comparison will include a logistic regression model where dependent variable will be the attainment of the Primary endpoint (Yes/No) and independent variables will be the group of treatment and the stratification factor [PSI categorical (≤90 or >90)]. For patients who are alive by Day 4 but any of the components is missing, the last observation carried forward (LOCF) method will be implemented for the missing values. Any p-value less than 0.05 will be considered statistically significant

An example of the table to be presented is the following:

Table: Logistic Regression model for the attainment of the Primary endpoint (ITT set)

Logistic Regression model	OR (95%CIs)	P-value
Clarithromycin treatment	x.xx (x.xx to x.xx)	x.xxx
PSI >90	x.xx (x.xx to x.xx)	x.xxx

A boxplot showing OR (95% CI) and p-value by group will be presented.

4.1.1 Post-hoc analysis of Primary Endpoint

An ordinal multivariate regression analysis model will also be implemented where the outcome will be the number of elements (0 to 3 elements) of the primary endpoint attained per patient. Dependent variables will be the treatment group (Clarithromycin or Placebo) and the PSI categorical (≤90 or >90). The final parameter estimates table will show all variables included in the model (OR, 95% CI and p-value). The alpha level will be 5%.

Table: Ordinal Logistic Regression model for the number of elements of the primary endpoint attained (ITT set)

Ordinal Logistic Regression model	OR (95%CIs)	P-value
Clarithromycin treatment	x.xx (x.xx to x.xx)	x.xxx
PSI >90	x.xx (x.xx to x.xx)	x.xxx

A boxplot showing the attainment of individual elements of the primary endpoint by group OR (95% CI) and p-value, a stacked boxplot as well as heatmaps showing the attainment of the elements (0 to 3 elements) of the primary endpoint by group will be presented. Any p-value less than 0.05 will be considered statistically significant

4.1.2 Sensitivity Analysis

Two sensitivity analyses are provisioned:

- One multivariate logistic regression analysis introducing as co-variates any baseline variables (Table Baseline Characteristics of Participants in the REACT Trial) which are statistically different between the two groups. Any p-value less than 0.05 will be considered statistically significant

Table Sensitivity analysis: Multivariate Logistic Regression model (ITT set)

Multivariate Logistic Regression model	**OR (95%CIs)**	**P-value**
Clarithromycin treatment	x.xx (x.xx to x.xx)	x.xxx
Statistically significant variable(s)	x.xx (x.xx to x.xx)	x.xxx

- One logistic regression analysis analyzing only the patient population with positive microbiology. Any p-value less than 0.05 will be considered statistically significant

Table Sensitivity analysis: Univariate Logistic Regression model for patient population with positive microbiology

Multivariate Logistic Regression model	**OR (95%CIs)**	**P-value**
Clarithromycin treatment	x.xx (x.xx to x.xx)	x.xxx

A boxplot showing the attainment of the primary endpoint for patients with positive and negative microbiology by group will also be presented.

4.2 Key secondary efficacy endpoints

4.2.1 Time until progression into organ dysfunction by Day 28

The time to development of new organ dysfunction by Day 28 will be evaluated using Cox regression analysis showing the HR (95% CI) and p-value of Clarithromycin + SoC versus Placebo + SoC group.

Day 1 (starting point) will be the date of enrolment. For patients developing more than one organ dysfunction, the time to the first organ dysfunction will be counted.

A Kaplan-Meier curve will be presented to compare Clarithromycin + SoC versus Placebo + SoC. The number of patients who experienced the event will be presented as n (%; 95% CI) for each treatment group. Below the x-axis, a table will display the number of patients at risk in each treatment group at each specified time point during the follow-up period.

The hazard ratio (HR), 95% confidence interval (CI), and p-value for the comparison between Clarithromycin + SoC and Placebo + SoC will be estimated using a univariate Cox proportional hazards regression model. The adjusted HR, 95% CI, and p-value, based on a multivariate Cox proportional hazards regression model including the PSI category (≤90 vs >90) as a covariate, will also be presented. Any p-value less than 0.05 will be considered statistically significant

4.2.2 Time until development of new sepsis by Day 28

The time to development of new sepsis by Day 28 will be evaluated using Cox regression analysis showing the HR (95% CI) and p-value.

Day 1 (starting point) will be the date of enrolment. For patients developing more than one sepsis episode, the time to the first sepsis episode will be counted.

A Kaplan-Meier curve will be presented to compare Clarithromycin + SoC versus Placebo + SoC. The number of patients who experienced the event will be presented as n (%; 95% CI) for each treatment group. Below the x-axis, a table will display the number of patients at risk in each treatment group at each specified time point during the follow-up period.

The hazard ratio (HR), 95% confidence interval (CI), and p-value for the comparison between Clarithromycin + SoC and Placebo + SoC will be estimated using a univariate Cox proportional hazards regression model. The adjusted HR, 95% CI, and p-value, based on a multivariate Cox proportional hazards regression model including the PSI category (≤90 vs >90) as a covariate, will also be presented. Any p-value less than 0.05 will be considered statistically significant

4.3 Secondary efficacy endpoints

4.3.1 Clinical success at the Test of Cure (TOC) visit

The test-of-cure (TOC) visit is taking place seven days from end of randomized treatment (Day 14). Clinical success is defined as complete resolution of the respiratory symptoms score (RSS). The two arms of treatment will be compared for the primary endpoint first using the Fisher exact test, where any 2-sided p-value less than 0.05 will be considered statistically significant. Then definite comparison will include group of treatment and the PSI categorical (≤90 or >90) variable.

A boxplot by group of treatment will also be displayed showing the OR (95% CI) and p-value. Any p-value less than 0.05 will be considered statistically significant

4.3.2 Up-Escalation of Antimicrobial Treatment

Up-escalation of antimicrobial treatment is defined as a change from the baseline SoC antibiotic. The comparison between the treatment groups will be done by Cox regression analysis from a multivariate regression model including the PSI stratification variable as a dependent variable.

A Kaplan-Meier curve will be presented to compare Clarithromycin + SoC versus Placebo + SoC. The number of patients who experienced the event will be presented as n (%; 95% CI) for each treatment group. Below the x-axis, a table will display the number of patients at risk in each treatment group at each specified time point during the follow-up period.

The hazard ratio (HR), 95% confidence interval (CI), and p-value for the comparison between Clarithromycin + SoC and Placebo + SoC will be estimated using a univariate Cox proportional hazards regression model. The adjusted HR, 95% CI, and p-value, based on a multivariate Cox proportional hazards regression model including the PSI category (≤90 vs >90) as a covariate, will also be presented. Any p-value less than 0.05 will be considered statistically significant.

4.3.3 Alive hospital discharge until Day 28

Alive at hospital discharge until Day 28 (days) will be compared between the treatment groups using a Cox regression multivariate analysis model including the PSI stratification variable as a dependent variable. With regards to the time to alive at hospital discharge, for any patients who died before or at Day 28 while they were hospitalized, the time from Day 1 to date of death will be considered. For patients who will be alive at hospital discharge, the time to alive at hospital discharge (days) will be calculated as follows (date of discharge – date of admission) + 1.

A Kaplan-Meier curve will be presented to compare Clarithromycin + SoC versus Placebo + SoC. The number of patients who experienced the event will be presented as n (%; 95% CI) for each treatment group. Below the x-axis, a table will display the number of patients at risk in each treatment group at each specified time point during the follow-up period.

The hazard ratio (HR), 95% confidence interval (CI), and p-value for the comparison between Clarithromycin + SoC and Placebo + SoC will be estimated using a univariate Cox proportional hazards regression model. The adjusted HR, 95% CI, and p-value, based on a multivariate Cox proportional hazards regression model including the PSI category (≤90 vs >90) as a covariate, will also be presented. Any p-value less than 0.05 will be considered statistically significant

4.3.4 Achievement of more than 50% reduction from baseline SOFA score at the End-of-Treatment (EOT) visit

EOT visit will be Day 8 (Visit 8) from the initiation of treatment (Day 1).
The number and percentage of patients with at least 50% decrease of SOFA score by EOT visit per group of treatment will be presented [n (%); 95% CI]. The Fisher's exact p value of the treatment groups comparison will also be presented. In addition, the OR (95% CI) and p-value from a univariate logistic regression model will be presented. The multivariable logistic regression model will also be presented including group of treatment and the PSI categorical (≤90 or >90) variable.
A boxplot by group of treatment will also be displayed showing the univariate logistic regression OR (95% CI) and p-value. Any p-value less than 0.05 will be considered statistically significant

4.3.5 Achievement of the predefined score of improvement of CAP-associated immune dysregulation at the EOT visit

The attainment of the score of improvement in CAP-associated immune dysregulation at EOT (Yes/No) is defined as the combination of: (Plasma PCT on EOT decreased by at least 80% from baseline PCT of screening or PCT below 0.25 ng/ml) AND ([plasma IL-10 at EOT decreased by at least 25% compared to IL-10 of visit 1 or IL-10 below the lower limit of detection] or [the IL-8 to IL-10 ratio of EOT decreased less than 15% from the IL 8 to IL-10 ratio of visit 1]). The following rule applies for the variables:

$$PlasmaPCTdiff = \frac{\text{PlasmaPCTday8} - \text{PlasmaPCTscreening}}{\text{PlasmaPCTscreening}} * 100$$

A new categorical variable [pctruleday8 (Yes/No)] will then be created. The variable will be equal to “Yes” if $Plasma\ PCT$ is decreased by at least 80% or Plasma PCT on Day 8 is below 0.25 ng/ml. If none of the conditions are satisfied, then “pctruleday8” is “No”.

$$\text{IL10}\ diff = \frac{\text{IL10day8} - \text{IL10day1}}{\text{IL10day1}} * 100$$

A new categorical variable [il10day8rule (Yes/No)] will then be created. The variable will be equal to “Yes” if $\text{IL10}\ diff$ is decreased by at least 25% or plasma IL10day8 is below the lower limit of detection. If none of the conditions are satisfied, then “il10day8rule” will be “No”.

$$\text{IL8/IL10 ratio} = \frac{\frac{\text{IL8}}{\text{IL10}}\text{day8} - \frac{\text{IL8}}{\text{IL10}}\text{day1}}{\frac{\text{IL8}}{\text{IL10}}\text{day1}} * 100$$

A new categorical variable [il8il10decrease15day8 (Yes/No)] will then be created. The variable will be equal to “Yes” if $\frac{\text{IL8}}{\text{IL10}}\text{ratio}\ is\ decreased$ by at least 15% from the IL8/IL10 ratio ratio of Day 1 or “No” otherwise.

Condition C at EOT is considered satisfied only if the following apply:

[(Variable pctruleday8 = “Yes” and variable il10day8rule = “Yes”) or variable il8il10decrease15day8 = “Yes”].

The number and percentage of patients who attained the score of improvement in CAP-associated immune dysregulation at EOT will be presented [n (%); 95% CI]. The Fisher's exact p value of the treatment groups comparison will also be presented. In addition, the OR (95% CI) and p-value from a univariate logistic regression model will be presented. The multivariable logistic regression model will also be presented including group of treatment and the PSI categorical (≤90 or >90) variable.

A boxplot by group of treatment will also be displayed showing the univariate logistic regression OR (95% CI) and p-value. Any p-value less than 0.05 will be considered statistically significant

4.3.5.1 Additional analysis of achievement of the predefined score of improvement of CAP-associated immune dysregulation at the EOT visit

The achievement of the predefined score of improvement of CAP-associated immune dysregulation at the EOT visit will be further analysed in relation to the Primary endpoint.

Therefore, the following analysis of this endpoint will also be performed:

The following conditions will be evaluated for EOT visit as well. These are the following:

Condition A at EOT:
If RSS at EOT is decreased by at least 50% from baseline and the patient hasn't developed any new symptoms then Condition A at EOT will be considered satisfied.

Condition B at EOT:
If SOFA at EOT is decreased by at least 30% from baseline then Condition B at EOT will be considered satisfied.

Condition C at EOT:
[(Variable pctruleday8 = "Yes" and variable il10day8rule = "Yes") or variable il8il10decrease15day8 = "Yes"].

Patients meeting ALL three conditions (Condition A at EOT & Condition B at EOT & Condition C at EOT) will be considered as succeeding this endpoint.

Patients dying prior to Day 8 will be considered as failing this endpoint.

The two arms of treatment will be compared for this endpoint using a logistic regression model where dependent variable will be the attainment of the endpoint (Yes/No) and independent variables will be the group of treatment and the stratification factor [PSI categorical (≤90 or >90)]. For patients who are alive by Day 8 (EOT) but any of the components is missing, the last observation carried forward (LOCF) method will be implemented for the missing values.

An example of the table to be presented is the following:

Table: Logistic Regression model for the attainment of the endpoint at the EOT (ITT set)

Logistic Regression model	**OR (95%CIs)**	**P-value**
Clarithromycin treatment	x.xx (x.xx to x.xx)	x.xxx
PSI >90	x.xx (x.xx to x.xx)	x.xxx

A boxplot showing OR (95% CI) and p-value by group will be presented. Any p-value less than 0.05 will be considered statistically significant

4.3.6 Change in cytokine production by peripheral blood mononuclear cells (PBMCs) on Day 4 compared with Visit 1

The cytokines measured in the supernatants of PBMCs isolated from patients at baseline and on day 4 are TNFα, IL-6 and IL-10. The ratio of TNFα to IL-10 and of IL-6 to IL-10 will be calculated as an expression of the pro-inflammatory versus anti-inflammatory cytokines. The number and percentage of patients who attained at least 2-fold increase of the TNFα/IL-10 and/or IL-6/IL-10 ratio in the supernatants of PBMCs on day 4 from day 1 (Yes/No) will be presented [n (%); 95% CI]. The Fisher's exact p value of the treatment groups comparison will also be presented. In addition, the OR (95% CI) and p-value from a

univariate logistic regression model will be presented. Any p-value less than 0.05 will be considered statistically significant

4.3.7 Association of 28-day mortality with the score of improvement of CAP-associated immune dysregulation

The association of 28-day mortality (Yes/No) with the score of improvement of CAP-associated immune dysregulation will be examined by a multivariable logistic regression model where treatment (Clarithromycin versus Placebo) and Improvement of CAP-associated immune dysregulation (Yes vs No). The OR (95%Cis) and p-value should be presented. Any p-value less than 0.05 will be considered statistically significant

The table to be presented should be the following:

Table: Association of 28-day mortality with the score of improvement of CAP-associated immune dysregulation

Multivariable Logistic Regression model	**OR (95%CIs)**	**P-value**
Clarithromycin treatment	x.xx (x.xx to x.xx)	x.xxx
Improvement of CAP-associated immune dysregulation	x.xx (x.xx to x.xx)	x.xxx

4.3.8 Association of 90-day mortality with the score of improvement of CAP-associated immune dysregulation

The association of 90-day mortality (Yes/No) with the score of improvement of CAP-associated immune dysregulation will be examined by a multivariable logistic regression model where treatment (Clarithromycin versus Placebo) and Improvement of CAP-associated immune dysregulation (Yes vs No). The OR (95%Cis) and p-value should be presented. Any p-value less than 0.05 will be considered statistically significant

The table to be presented should be the following:

Table: Association of 90-day mortality with the score of improvement of CAP-associated immune dysregulation

Multivariable Logistic Regression model	OR (95%CIs)	P-value
Clarithromycin treatment	x.xx (x.xx to x.xx)	x.xxx
Improvement of CAP-associated immune dysregulation	x.xx (x.xx to x.xx)	x.xxx

4.4 Analyses not provisioned in the protocol

Impact of corticosteroid treatment on the primary endpoint

Enrolled patients will be split into subgroups under treatment with and without systemic corticosteroids. The primary endpoint will be compared separately between the SoC and placebo group and the SoC and clarithromycin treated patients for each subgroup by the Fisher exact test; ORs and 95% Cis are calculated. Any 2-sided p-value will be considered statistically significant.

Predictive and prognostic performance of biomarkers

This will be done to validate: a) the predictive performance of SCORE2 for increased suPAR; b) the predictive performance of CURB65 for the combination of PCT and suPAR/SCORE2; c) the prognostic performance of PCT/suPAR for unfavorable outcome; and d) the prognostic performance of PCT/SCORE2 for unfavorable outcome.
Unfavorable outcome will be any of: a) progression into organ dysfunction the first 28 days; b) development of new sepsis the first 28 days; and c) death the first 28 days.
Population analyzed for (a) and (b) will be the total screened. Population analyzed for (c) and (d) will be patients failing screening and patients randomized to the SoC and placebo group

For (a) and (b), the following Table example will be followed:

	Biomarker (+)	**Biomarker (-)**	**Total**
Proxy (+)	a Sensitivity= a/(a + b) PPV= a/(a + c)	c	a + c
Proxy (-)	b	d Sensitivity= d/(c + d) NPV= d/(b + d)	b + d
Total	a + b	c + d	a + b + c + d

where biomarker will be suPAR in the case of (a); and the combination of PCT and suPAR/SCORE2 in the case of (b); and proxy will be SCORE2 in the case of (a); and CURB65 in the case of (b)

For (c) and (d), the following Table example will be followed:

	Unfavorable outcome (+)	**Unfavorable outcome (-)**	**Total**
Proxy (+)	a Sensitivity= a/(a + b) PPV= a/(a + c)	c	a + c
Proxy (-)	b	d Sensitivity= d/(c + d) NPV= d/(b + d)	b + d
Total	a + b	c + d	a + b + c + d

where proxy will be the combination of PCT and suPAR in the case of (a); and PCT and SCORE2 in the case of (b)

28-day and 90-day mortality

The number and percentage of patients who died by 28-day and by 90-day will be presented [n (%); 95% CI]. The Fisher's exact p value of the treatment groups comparison will also be presented. In addition, the HR (95% CI) and p-value from a univariate Cox-regression model will be presented. The adjusted HR (95% CI) and p-value from multivariable Cox-regression model will also be presented for group of treatment where the PSI categorical (≤90 or >90) variable will be a co-variate. Missing values for the 28-day and 90-day mortality will be imputed by last observation carried forward. Any p-value less than 0.05 will be considered statistically significant

Time points should start from the date of enrolment.

A Kaplan-Meier curve will be presented to compare Clarithromycin + SoC versus Placebo + SoC. The number of patients who experienced the event will be presented as n (%; 95% CI) for each treatment group. Below the x-axis, a table will display the number of patients at risk in each treatment group at each specified time point during the follow-up period.

The hazard ratio (HR), 95% confidence interval (CI), and p-value for the comparison between Clarithromycin + SoC and Placebo + SoC will be estimated using a univariate Cox proportional hazards regression model. The adjusted HR, 95% CI, and p-value, based on a multivariate Cox proportional hazards regression model including the PSI category (≤90 vs >90) as a covariate, will also be presented. Any p-value less than 0.05 will be considered statistically significant

5. Toxicity/safety

All AEs will be coded using the MedDRA dictionary (version 28.1). The non-serious (TEAEs) and serious treatment emergent AEs (sTEAEs) will be summarized overall and by treatment group. The serious treatment emergent AEs (sTEAEs) will be defined as TEAEs regarded by the investigator as Serious =" Yes". The sTEAEs will be summarized overall and by SOC (System Organ Class) and PT (Preferred Term) as well as classified by Outcome (Recovery/Non recovery/Recovery with consequences/ Recovering/Death/ Unknown), Severity (Mild/Moderate/Severe) and by Relationship (Probably Related/Possibly Related/Probably not Related/Unrelated) to study drug.

The sTEAEs will be summarized overall and by SOC (System Organ Class) and PT (Preferred Term) as well as classified by Outcome (Recovery/Non recovery/Recovery with consequences/ Recovering/Death/ Unknown), Severity (Mild/Moderate/Severe) and by Relationship (Probably Related/Possibly Related/Probably not Related/Unrelated) to study drug. The non-serious TEAEs will be summarized overall and by SOC (System Organ Class) and PT (Preferred Term) as well as classified by Outcome (Recovery/Non recovery/Recovery with consequences/ Recovering/Death/ Unknown) and by Relationship (Probably Related/Possibly Related/Probably not Related/Unrelated) to study drug.
The two arms of treatment will be compared for the primary endpoint first using the Fisher exact test, where any 2-sided p-value less than 0.05 will be considered statistically significant.

Example of the table:

Table: Non-Serious TEAEs by System Organ Class and Preferred Term classified by relationship to study drug (Safety set)

	Placebo + SoC (n=xxx)	**Clarithromycin + SoC (n=xxx)**	**Total (n=xxx)**	**P-value***
Any Non-Serious TEAE	**xxx (xx.x%)**	**xxx (xx.x%)**	**xxx (xx.x%)**	**x.xxx**
Probably Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	**x.xxx**
Possibly Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	**x.xxx**
Probably not Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	**x.xxx**
Unrelated	xx (x.x%)	xx (x.x%)	xx (x.x%)	**x.xxx**
Investigations	**xxx (xx.x%)**	**xxx (xx.x%)**	**xxx (xx.x%)**	**x.xxx**
Alanine aminotransferase increased	**xxx (xx.x%)**	**xxx (xx.x%)**	**xxx (xx.x%)**	**x.xxx**
Probably Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	
Possibly Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	
Probably not Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	
Unrelated	xx (x.x%)	xx (x.x%)	xx (x.x%)	
Aspartate aminotransferase increased	**xxx (xx.x%)**	**xxx (xx.x%)**	**xxx (xx.x%)**	**x.xxx**
Probably Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	
Possibly Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	
Probably not Related	xx (x.x%)	xx (x.x%)	xx (x.x%)	
Unrelated	xx (x.x%)	xx (x.x%)	xx (x.x%)	

*Fisher's Exact test.

For patients with multiple AEs worst relation has been counted

For each type of event, the table will show the number (%) of patients. One patient can appear in more than one row.