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

STATISTICAL ANALYSIS PLAN

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LIST OF ABBREVIATIONS	
AE	adverse event
APACHE	Acute physiology and chronic health evaluation
CCI	Charlson's comorbidity index
CI	confidence interval
ITT	Intent-to-treat
OR	odds ratio
PCT	Procalcitonin
RCT	randomized clinical trial
SD	standard deviation
SOFA	sequential organ failure assessment
PBMCs	Peripheral blood mononuclear cells

BACKGROUND

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

STUDY DESIGN AND ANALYSIS POPULATION

This is a prospective randomized placebo-controlled phase 2 clinical trial in a total number of 5 study sites. The analysis of the trial is based on the principles of intent-to-treat (ITT) population. ITT is defined as patients randomized to one arm of treatment. Patients who request removal of consent and of data after randomization are removed from ITT. According to the sample size calculation of the study protocol, it is anticipated that 60 patients will be enrolled.

PATIENT BASELINE DEMOGRAPHICS

Baseline demographics (i.e. baseline characteristics of the study population before start of the study drug) will be expressed differently for quantitative variables and for qualitative variables. Quantitative variables will analyzed by Kolmogorov-Smirnov's statistics if they meet normal distribution or not. In case they follow normal distribution, they will be expressed as mean +/- SD. In case they follow non-normal distribution, they will be expressed as median and first and third quartile of distribution. Quantitative variables following normal distribution will be compared between groups by the Student's t-test; variables following non-normal distribution will be compared by the Mann-Whitney U test. Qualitative variables will be compared between groups by the Fisher's exact test.

ANALYSIS FOR THE STUDY ENDPOINTS

Primary study endpoint

According to the study protocol, the primary efficacy study endpoint This is the progression into organ dysfunction which is a composite endpoint. Patients who meet any of the following are considered to meet this endpoint: i) increase of SOFA score by 2 or more points from day 1 (before start of the study drug) until day 7; ii) death by

day 90. The two groups of treatment are compared regarding the achievement of the primary endpoint or not by the Fisher's exact test. The odds ratio (ORs) and 95% confidence intervals (CIs) are calculated.

The analysis of the primary endpoint is further confirmed by one step-wise logistic regression model: achievement of the primary endpoint is the dependent variable and group of treatment (placebo/anakinra) and APACHE II, CCI and SOFA are the independent variables. ORs and 95% CIs are calculated.

Any p-value less than 0.05 is considered statistically significant.

Secondary endpoints

Change of SOFA score over all days of follow-up

This is analyzed as the number of patients per group which are experiencing at least 2 points decrease of SOFA score from baseline. The two groups of treatment are compared by the Fisher's exact test. The odds ratio (ORs) and 95% confidence intervals (CIs) are calculated according to Mantel and Haenszel.

Incidence of specific organ dysfunction by day 28

The frequency of each organ dysfunction, as defined in the study protocol, is compared between groups by the Fisher's exact test. The odds ratio (ORs) and 95% confidence intervals (CIs) are calculated according to Mantel and Haenszel.

Mortality by day 28

This will be analyzed by plotting survival curves for patients allocated to placebo treatment and for patients allocated to Anakinra treatment by Kaplan-Meir and by comparisons using Cox regression analysis; one step-wise logistic model will be done where mortality is the dependent variable and group of treatment (placebo/anakinra) and APACHE II, CCI and SOFA are the independent variables. Hazard ratios and 95% CIs are calculated.

Mortality by day 90

This will be analyzed by plotting survival curves for patients allocated to placebo treatment and for patients allocated to Anakinra treatment by Kaplan-Meir and by comparisons using Cox regression analysis; one step-wise logistic model will be done where mortality is the dependent variable and group of treatment (placebo/anakinra)

and APACHE II, CCI and SOFA are the independent variables. Hazard ratios and 95% CIs are calculated.

Day until attenuation of sepsis-induced inflammation as defined by PCT measurements

The time until patients meet the PCT rule, defined in the protocol, will be compared between groups by Cox regression analysis; Hazard ratios (HRs) and 95% CIs are calculated.

Change of presepsin from baseline until day 10

The time until patients meet the experience at least 50% decrease of blood presepsin from baseline will be compared between groups by Cox regression analysis; Hazard ratios (HRs) and 95% CIs are calculated.

Comparison of change of cytokine function and endothelial dysfunction markers from baseline by days 4 and 7

Analysis will be done by two approaches. In the first approach, cytokine measured in the supernatants of PBMCs and endothelial dysfunction markers measured in the blood will be compared by the Mann Whitney U test. Then the frequency of patients of each group experiencing at least 30% change (either increase or decrease) of cytokine is PBMC supernatants from baseline will be compared by the Fisher's exact test. The odds ratio (ORs) and 95% confidence intervals (CIs) are calculated according to Mantel and Haenszel.

Comparative progression into organ dysfunction by day 10 (defined as in the primary endpoint) between patients who failed screening because of presepsin 350 pg/ml or less and patients who were enrolled in the study and were allocated to Treatment Arm 1

Comparisons are done by the Fisher's exact test. The odds ratio (ORs) and 95% confidence intervals (CIs) are calculated according to Mantel and Haenszel.

Comparative 28-day mortality between patients who failed screening because of presepsin 350 pg/ml or less and patients who were enrolled in the study and were allocated to Treatment Arm 1

Comparisons are done by Cox regression analysis; Hazard ratios (HRs) and 95% CIs are calculated.